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CENTER FOR INDOOR AIR RESEARCH
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APPLICATION FOR RESEARCH CONTRACT

1. PRINCIPAL INVESTIGATOR. NAME, TITLE, TELEPHONE # AND MAILING ADDRESS.

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2. PROJECT TITLE. Development of Inhalant Allergy and Asthma in Children

3. KEY WORDS. PLEASE PROVIDE THREE (3) KEY WORDS WHICH WILL BE USED AS REFERENCE HEADINGS. Wheezing, Allergy, Cotinine

4. INSTITUTION. NAME AND ADDRESS OF INSTITUTION RESPONSIBLE AND ACCOUNTABLE FOR DISPOSITION OF FUNDS AWARDED ON THE BASIS OF THIS APPLICATION.

(A) INSTITUTION: University of Virginia (B) STREET ADDRESS: P.O. Box 9003

(C) CITY: Charlottesville (D) STATE/ZIP: Virginia 22903

5. LOCATION. LIST LOCATION WHERE RESEARCH WILL BE CONDUCTED *IF OTHER THAN INSTITUTION IDENTIFIED IN #4 ABOVE*.

(A) Same

(B)

6. INCLUSIVE DATES AND TOTAL COSTS OF THIS SPECIFIC PROJECT RELATED TO EACH 12 MONTH PERIOD IF MORE THAN ONE YEAR IS REQUIRED TO COMPLETE PROJECT. SUMMARIZE FROM BUDGET PAGE, ITEM 12(i). IT MUST BE UNDERSTOOD THAT AWARDS FOR 2ND AND 3RD PERIODS ARE DEPENDENT ON CENTER APPROVAL OF CONTINUATION APPLICATION.

	INCLUSIVE DATE			TOTAL COST
(A) 1ST 12 MONTH PERIOD	1/1/94	THRU	12/31/94	$
(B) 2ND 12 MONTH PERIOD IF REQUIRED	1/1/95	THRU	12/31/95	$ REDACTED
(C) 3RD 12 MONTH PERIOD IF REQUIRED	1/1/96	THRU	12/31/96	$

7. INSTITUTIONAL OFFICER. NAME, TITLE AND TELEPHONE NUMBER OF INDIVIDUAL AUTHORIZED TO SIGN FOR THE INSTITUTION IDENTIFIED IN #4 ABOVE. IT IS UNDERSTOOD THAT THE OFFICER, IN APPLYING FOR A CONTRACT, HAS READ AND FOUND ACCEPTABLE THE CENTER'S MANAGEMENT OF RESEARCH CONTRACTS AND CONTRACT ADMINISTRATION POLICY.

(A) NAME: D. Wayne Jennings (B) TITLE: Director, Office of Sponsored Programs

(C) TELEPHONE: (804) 924-4270 (D) SIGNATURE OF INSTITUTIONAL OFFICER: R. Wayne Jennings (E) DATE: 5/28/93

8. PRELIMINARY STUDIES*.
(A) FEASIBILITY OF PROPOSED RESEARCH
(B) QUALIFICATIONS OF INVESTIGATOR

9. EXPERIMENTAL PLAN*.
(A) DESIGN
(B) METHODS
(C) ANALYSIS OF DATA
(D) INTERPRETATION OF RESULTS
(E) TIMETABLE FOR THE INVESTIGATION
(F) LITERATURE CITED

10. AVAILABLE FACILITIES AND RESOURCES.

11. OTHER SUPPORT

* APPEND AS MUCH MATERIAL AS REQUIRED. TYPE, SINGLE SPACE, USE 8-1/2" X 11" WHITE PAPER AND LABEL EACH SHEET WITH NAME OF THE PRINCIPAL INVESTIGATOR IN THE UPPER RIGHT HAND CORNER AND PAGE NUMBER AT THE BOTTOM. CONSECUTIVELY NUMBER EACH ADDENDUM BEGINNING WITH PAGE 5. DO NOT INSERT PAGES BETWEEN PAGES 1 AND 6; E.G., 2A, 2B, 3A, ETC. INCLUDE NINE COPIES AND AN ORIGINAL. IF SENDING PHOTOGRAPHS, INCLUDE 2 ORIGINAL SETS. NOTE: EACH OF THE NINE COPIES *MUST* BE PLACED IN A BINDER PER MAILING INSTRUCTIONS.

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13. **BIOGRAPHICAL SKETCH** of all professional personnel listed in 12(a). Append. Please include the following: Name, title, education, scientific field, major research interest, research and/or professional experience and publications. (Limit list of publications to the 20 most important and/or relevant.)

14. a) Are **HUMAN SUBJECTS** to be used in this research? X Yes _____ No
If yes, attach Institutional Review Board approval for procedures involving human subjects.

b) Are **LABORATORY ANIMALS** to be used in this research? X Yes _____ No
If yes, attach Institutional Animal Care and Use Committee approval for procedures involving animals.

15. **SIGNATURE OF PRINCIPAL INVESTIGATOR**: It is understood that the applicant in applying for a Contract has read and found acceptable the Statements of Policy and Terms Under Which Project Contracts Are Made appearing in the application package.

Peter W. Hagamann — 9/24/93

Signature of Principal Investigator — Date

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8. SPECIFIC AIMS, BACKGROUND AND SIGNIFICANCE, AND PRELIMINARY STUDIES

A. SPECIFIC AIMS

Over the past decade, hospitalization rates have risen substantially for wheezing children during the first four years of life. The main objective of the proposal is based on the premise that a better understanding for 1) the relationship between IgE ab to inhalant allergens and other major pathogenic factors for wheezing and for 2) the identification of young children who are at risk for developing asthma will improve our management of these children significantly. The following studies are planned:

1)*Specific Aim 1*: Emergency Room (ER) Study. Children treated for wheezing in the Pediatric ER at the University of Virginia and controls will be enrolled into three age-matched groups (i.e. 2 to 23 months; 2 to 4 years; and 4 to 16 years). The evaluation of these children will include:

a) Measurements of IgE antibody: Serum IgE ab by RAST will be measured to dust mite, cat, cockroach, rye grass, and ragweed allergens, as well as to egg, milk, peanut, and soy. Total serum IgE levels will be measured as will IgE ab responses to the major Group I and Group II mite allergens.

b) Analysis of mediators and eosinophils in nasal secretions and blood: Tryptase from mast cells and eosinophil cationic protein (ECP) from eosinophils will be measured in nasal secretions, as will kinins, which have been shown to be elevated in nasal secretions during rhinovirus infections. Eosinophils will be enumerated in nasal secretions and blood and serum ECP will also be measured.

c) Assessment of other risk factors for wheezing: Nasal secretions will be obtained for viral cultures (rhinovirus, adenovirus, parainfluenza virus, influenza virus A and B, enterovirus, and RSV),detection of RSV antigen, and analysis for rhinovirus RNA by polymerase chain reaction (PCR). Saliva for determinations of cotinine, a metabolic derivative of nicotine, will also be obtained from each patient.

d) Analysis of history and physical findings: Each patient's history for allergic symptoms, family history for allergy, medications used, and exposure to tobacco smoke will be recorded. Physical findings will be documented and the patient's socioeconomic status assessed.

2)*Specific Aim 2*: Prospective Study. Infants (2 to 18 months old) who we anticipate will vary in their risk for developing inhalant allergy and/or asthma will be enrolled and assessed periodically in the allergy clinic at ages 2,3 and 4 years. Wheezing infants will be enrolled into three groups and will include those: a) treated in the emergency room, b) admitted to the hospital with O_2 saturations less than 90%, and c) evaluated in the allergy clinic and shown to have an atopic parent (some infants will also be sensitized to food allergen). Infants with atopic dermatitis (AD), control patients without a history of wheezing or AD, and controls from allergic parents will also be enrolled. Evaluations will include:

a) Measurements of IgE antibody: The assessments will be the same as above and include skin tests for the same allergens analyzed by RAST.

b) Analysis of mediators and eosinophils in nasal secretions and blood: Assessments of nasal mediators and eosinophils as described above for acutely wheezing patients are planned at the time of enrollment. Blood eosinophils, serum ECP, and nasal eosinophils will also be assessed periodically.

c) Assessment of exposure to indoor allergens and tobacco smoke: Dust samples will be

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collected from homes of patients at the time of enrollment and annually each fall. Concentrations of dust mite, cat, and cockroach allergens will be measured in these samples using two-site monoclonal antibody based assays. Results will be analyzed in relation to the development of IgE ab to the same allergens in these patients. Saliva for cotinine determinations will be obtained from patients and family members at the time of annual home visits.

d) Analysis of history and physical findings: Patients' histories and physical findings will be recorded at the time of enrollment and at annual visits to clinic. Questionnaires to assess the home environment and the socioeconomic background of patients will be completed during annual home visits. Parents will also be evaluated for sensitization to inhaled allergens by skin test and RAST.

Results of these studies are expected to (1) improve the diagnosis and management of inhalant allergy and asthma in young children, (2) improve our understanding for the relationship between bronchiolitis and asthma and the prognosis of those who begin to wheeze as infants, and (3) clarify the relationship between wheezing in early childhood and environmental risk factors which can be modified.

2. BACKGROUND AND SIGNIFICANCE

The strong association between immediate hypersensitivity to inhaled allergens and asthma in children and young adults is well recognized. Between 75-85 percent of children with asthma have positive skin test responses to common aeroallergens, and exposure to these allergens has been shown to lead to acute exacerbations of wheezing (17-19). In addition, allergen avoidance (e.g. reduction in mite allergen exposure) has been shown to lead to significant improvements in symptoms and airway hyperresponsiveness (20,21). As a result, environmental controls to reduce exposure, particularly to indoor allergens, are now strongly recommended for the management of children with allergic asthma.

During the last decade, the morbidity and severity of symptoms associated with wheezing in childhood has increased. As judged by hospitalization rates, however, the morbidity and severity of symptoms rose most dramatically for children less than age 4 (7), and in this age group the relationship between aeroallergen hypersensitivity and other risk factors for wheezing remains poorly understood. When children less than 2 years of age present with wheezing, they are often given the diagnosis of "bronchiolitis". The term bronchiolitis is generally used to describe wheezing in infancy commonly associated with viral respiratory tract infection, particularly due to respiratory syncytial virus (RSV). More recent studies indicate that infants who have a greater risk for wheezing with infection are those who 1) have decreased total airway conductance at birth, 2) are exposed to maternal tobacco smoke, and/or 3) are exposed to respiratory tract pathogens more frequently at day care (22-25). Considerable confusion still exists, however, about the etiology of bronchiolitis and the prognosis of those who present with this disorder for developing asthma (5). Most likely, wheezing in infancy includes different groups of children, some of whom are presenting early in life with asthma. For this reason, studies over the past decade are in conflict as to the relevance of atopy in bronchiolitis and whether bronchiolitis, by itself, leads to recurrent wheezing later in childhood. There are several studies, including recent investigations, which suggest that bronchiolitis is often followed by recurrent wheezing, long term respiratory sequelae, and pulmonary function abnormalities (26-30). In addition, an IgE-mediated mechanism for wheezing in infancy has been proposed based on the detection of IgE ab specific for viral (RSV and parainfluenza virus) antigens in nasopharyngeal secretions from hospitalized children with bronchiolitis (8,9). In contrast, others view bronchiolitis to be caused predominantly by virus

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infection, and in our experience, many children with bronchiolitis outgrow their wheezing as toddlers and do not develop asthma. This observation, however, has not been confirmed in any prospective studies, nor is it clear what proportion of children with bronchiolitis this represents.

Currently, the same medications needed to treat asthma are used to manage bronchiolitis. Although symptoms do get better in some, most wheezing infants show little or no improvement. Therefore, the efficacy of asthma medications (particularly corticosteroids, theophylline, and nebulized cromolyn) remains controversial which further adds to the confusion about the pathogenesis of bronchiolitis and its relationship to asthma. Usually, anatomical factors, including the small caliber of lumens in the middle airways are cited as reasons why infants who wheeze do not respond well to these medications. However, there is little information about the pathogenic factors which lead to airway obstruction in bronchiolitis other than histologic observations (e.g. necrosis of the respiratory epithelium and peribronchial lymphocyte infiltrations) or reports that IgE ab to virus and mediators from mast cells and eosinophils might be involved (8,12,13,31). An important objective of our research is to clarify what proportion of wheezing infants are atopic and, therefore, may either be asthmatic or at risk for developing asthma. In addition, by analyzing ECP, tryptase, and kinins in nasal secretions from these infants and, for comparison, from older wheezing children, a better understanding for the pathophysiology of bronchitis will result from this research.

The objective assessment of IgE ab to allergens, viral infection, and tobacco smoke exposure in our ER study is the most comprehensive analysis of acute wheezing episodes in children currently available. Clear differences were apparent for young children under the age of 4 when examining the prevalence of these risk factors with respect to age. First, under age 2, a high rate of virus infection, especially RSV, was evident in wheezing children, who also had a low prevalence of IgE ab to inhaled and food allergens. In addition, among wheezing infants exposed to tobacco smoke at home high salivary cotinine levels were common. The levels detected were indicative of heavy smoke exposure (32), which others have shown leads to more frequent lower respiratory tract infection and wheezing during early childhood (24,33). Second, wheezing in the majority of children ages 2 to 4 already had pathogenic characteristics of asthma (e.g. IgE ab to inhaled and/or food allergens, elevated total IgE levels, and nasal and peripheral blood eosinophilia), and results from our research indicate that improvements in the diagnosis and management of wheezing in this age group can be achieved.

Currently, information regarding the evolution of immune responses to allergens during early childhood is limited to prospective studies of infants born to atopic parents. Based on skin test and RAST analyses, these studies indicate that IgE ab to food allergens are detected in up to 30% of these children during the first two years of life and a few of these infants also develop atopic dermatitis (3,4). In contrast, not many of these children developed IgE ab to inhalant allergens prior to age two. It is also clear from these studies that, even though a family history for allergy increases the likelihood that a child may become allergic, the family history by itself has a low screening efficiency for atopy. This problem has also been pointed out by others (34). For example, in one of these studies only 52% of the children had become sensitized to aeroallergens by age 11 and only one-third of this cohort developed asthma (4). In none of the prospective studies, however, were parents tested for their sensitization to inhalant or food allergens to substantiate the family history.

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Atopic dermatitis

An initial objective for our research was to determine whether the delay in the development of IgE ab to inhaled allergens also applies to infants who present with symptoms to our clinic, most of whom have either AD, or chronic or recurrent wheezing. Several studies of infants with AD have shown that many of these children have immediate hypersensitivity to food allergens, and this relationship has been confirmed in double-blind placebo controlled food challenges (35,36). In addition, there is good evidence that infants who present with AD have an increased risk for developing respiratory allergy (allergic rhinitis and asthma) later in childhood (37). In keeping with this risk, we have observed that infants with moderate to severe AD can develop IgE ab to aeroallergens well before age 2 (38). Some of these patients became sensitized before age one and the majority of the IgE ab responses were to indoor allergens (mite and cat). The implication of these results is that reductions in allergen exposure during infancy may be prudent to delay the development or minimize symptoms later on. It is unlikely, however, that AD in infancy will identify a large proportion of the children who will develop asthma since the prevalence of AD in the United States is estimated to be 1 to 2% as compared to the prevalence of asthma during childhood which is estimated to be about 7 to 8%. Consistent with these statistics, few of the children whom we see in our clinic for asthma and only 10% of patients with asthma from our ER Study have had a history of AD.

Young children with a positive skin test for IgE ab to egg, and possibly milk, have also been reported to have an increased risk for allergic respiratory disease and asthma, even in the absence of AD (39,40). The value of testing with egg, milk, and other common food allergens will be examined in our prospective studies. Results of skin tests for foods in our clinic together with measurements of serum IgE ab to foods in the ER Study already indicate that these tests can improve the efficiency of identifying atopic individuals prior to age 4 (41).

Other markers for atopy during infancy have also been investigated. Despite initial enthusiasm, the predictive value of an elevated cord blood IgE level ($\geq$ 1 IU/ml) is too low to be recommended as a routine screening test for atopy (34,42). Similarly, although total IgE levels have been shown to be higher in children from atopic families during the first 2 years of life, the range of levels in these children have been shown to overlap significantly with levels from non-atopic children and, therefore, limits its use as a sole predictor of atopic disease during early childhood (43). Thus, it is unlikely that a single marker for atopy will be sufficient for determining which infants might benefit from early allergy prevention. It is also unclear that a single marker for atopy will be discovered through genetic linkage analysis. Nor is it clear that a genetic marker(s) will be able to distinguish between infants who will become sensitized and develop symptoms early in life from those who become sensitized but do not develop symptoms during their childhood or adult years. Consequently, screening all neonates and infants on a routine basis may not be feasible. Therefore, a more practical approach in our studies will be to learn which children are the best candidates for early allergy screening and to examine combinations of tests (e.g. a positive test for IgE ab to food allergen in an infant and/or IgE ab to inhalant allergen in serum from a parent) as indicators for the development of allergic respiratory disease. From these studies, the atopic characteristics and other risk factors for symptoms in children who present with asthma early in life will become apparent. It is these children who present early in life who are reported to have a worse prognosis for symptoms that are difficult to manage (37) and who are most likely to benefit from early environmental controls (e.g. reduced exposure to allergens and tobacco smoke) in order to reduce the frequency and severity of their symptoms.

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C. PRELIMINARY STUDIES

Results from the first 156 patients enrolled in the emergency room study of wheezing children have been described in two manuscripts: "Risk factors for acute wheezing in infants and children: viral infection, passive smoke exposure, and IgE antibody to inhalant allergens" (Pediatrics, in press) and "Sensitization (IgE ab) to common food allergens in wheezing infants and children" (submitted). Data from patients enrolled a cross-sectional study of atopic dermatitis have also been submitted: "Early Childhood inhalant allergen sensitization in atopic eczema." All figures and tables summarizing data are printed at the end of this section.

1) Emergency Room (ER) Study:

The majority of infants seen in our Allergy Clinic are referred for wheezing to determine the role of IgE mediated hypersensitivity. Leading questions from referring physicians and parents about these patients are 1) at what age is an allergy evaluation indicated (many believe that tests for allergy in children who wheeze are not meaningful until after age three or four); 2) what will the prognosis of symptoms be in any given patient (will an infant outgrow his or her problems with recurrent or persistent wheezing, and when); and 3) how do we identify and manage those children who may be presenting with asthma early in life? In our clinic, we generally evaluate all infants referred for wheezing with prick skin tests for common food allergens (egg, milk, soy, wheat, and peanut). Other food allergens are added selectively as judged by the patient's symptoms and diet. Although this approach is often used by allergists to assess infants with eczema, this is not common practice for the evaluation of young children who wheeze. For inhalant allergens, we generally limit our tests to those which represent perennial exposures from birth on, (e.g. dust mite allergen, but also cat and cockroach allergens, if the history suggests that exposure is likely). We have observed that skin reactions to inhaled allergens are not often positive in children referred for wheezing before age 2. Although this observation is consistent with prospective studies of infants born to allergic parents (24,36,37), this has never been objectively assessed in infants who present with wheezing and for whom the risks for subsequent wheezing and asthma remain unclear.

The importance of the emergency room for our study is that we can enroll patients who are actively wheezing and require treatment. These patients also represent a non-selected population who are not being seen because allergy is suspected. In addition, we can examine the association between IgE ab responses and wheezing in these children together with other acknowledged risk factors for wheezing (e.g. viral respiratory tract infection and tobacco smoke exposure.)

In the ER, however, skin testing is not practical and is contra-indicated for actively wheezing subjects. However, *in vitro* tests for IgE ab (i.e. the radioallergosorbent test, or RAST) are established in our laboratory and permit measurements of serum IgE ab to more allergens than we use for skin testing infants in our clinic. Using the RAST test for inhalant allergens, we can measure IgE ab to two species of dust mite (*D. pteronyssinus* and *D. farinae*) and to cat, cockroach, rye grass, and ragweed. These assays were developed because they are the five inhalant allergens to which patients seen in our allergy clinics (pediatric and adult) react most frequently on skin tests (e.g. of the last 81 children with asthma who had positive skin tests in the pediatric clinic, 97% had reactions to one or more of these allergens and 88% had positive reactions to dust mite). In addition, the selection of these allergens for RAST is in keeping with allergens used in other published population surveys (38,39). To test for food allergens, we have developed RAST assays for egg, milk, soy, and peanut allergens which are common exposures during the first two years of life. These allergens were selected after reviewing skin

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test data from 63 food-sensitized children seen in our pediatric allergy clinic. These patients had a positive skin test (i.e. a wheal 3 mm greater than a saline control) to one or more foods, and 92% of these could be detected using a screen inclusive of these allergens which concurs with other reports (40). In addition, in 52 patients from the pediatric allergy clinic who had a positive food skin test, the percent concordance between prick test responses and our RAST assays using sera from these patients was very high (89%, 82%, 86%, and 85% for egg, milk, soy, and peanut, respectively). The rationale for developing RAST tests in our laboratory rather than utilizing commercial assays is that: 1) we use a micro-RAST procedure (41), which, in our laboratory, requires 17 μl of patient's serum for each test (to conserve blood from small children) as compared to 100 μl per test using commercial kits, and 2) the costs of commercial kits are considerably higher.

Thus far, 178 patients (ages 2 months to 16 years) have been enrolled in the ER Study (109 wheezing children and 69 controls). Demographic and other characteristics of the first 163 patients enrolled are shown in Table 1. Most (91%) were enrolled during the months of September through June. Similar to previous reports of wheezing in infancy (42), increased numbers of children under age 2 were treated in the ER from November through February when viral respiratory tract infections, particularly due to RSV, predominate. Older children were generally seen more frequently in the spring and fall months when exposures to outdoor and indoor (dust mite) inhaled allergens increase and viral respiratory tract infections, especially rhinovirus, are more prevalent.

a) Measurements of IgE antibody:

(1) IgE ab to inhaled allergens:

Among patients less than 2 years, only 2 of 23 (9%) had serum IgE ab to one or more of the inhaled allergens tested (i.e. were RAST positive) (Fig. 1A). Both positive patients were 22 months old and had IgE ab to dust mite allergen (one also had IgE ab to rye grass). Neither patient had atopic dermatitis (AD). The proportion of wheezing patients who were RAST positive increased to 35% in children ages 2 to 4 and, after age 4, 72% were RAST positive (compared to 30% of controls, $p < 0.001$). Most of the RAST positive patients (86%) had IgE ab to dust mite allergen (Fig.1B). Thus, the prevalence of sensitization (IgE ab) to inhaled allergens increased in children enrolled in this study after age 2, and was strongly associated with wheezing from age 4 on.

In general, skin tests have been reported to be more sensitive than RAST tests for judging sensitization to allergens (43). Skin tests, however, are contraindicated in actively wheezing patients. Thus far, RAST data for IgE ab to aeroallergens from patients enrolled in the ER Study are consistent with skin test observations from patients seen for wheezing in our clinic. Of the last 32 children less than age 2 who were referred, none had positive prick skin tests to inhaled allergens. Whether intradermal tests, (which use significantly more allergen), would detect sensitization in some of these young patients is an important question. We are beginning to do these tests using dust mite allergen for children under age 2 and for cat and cockroach allergens if exposure is suspected. For children over age 2, who were referred to our clinic for wheezing during the same time period, 40% of 35 patients ages 2 to 4 and 80% of 81 children over age 4 had positive skin reactions to one or more inhalant allergens.

(2) IgE ab to food allergens:

Immediate hypersensitivity to food allergens is strongly associated with symptoms of atop

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dermatitis. By contrast, much less is known about the relationship between food hypersensitivity and wheezing. Previous studies have focused on the incidence of wheezing induced by food challenges in patients who had other symptoms consistent with food allergy (e.g. AD)(44). Other studies have evaluated the occurrence of wheezing following food challenges in children referred for asthma and a history of food intolerance (45). However, the prevalence of sensitization to food allergens in a non-selected population of wheezing infants and older children has never been examined. Furthermore, because sensitization to food allergens can develop early in life, it would be very useful to know whether tests for foods could identify atopic infants who had not yet developed IgE ab to aeroallergens.

Sera from 163 children enrolled in the ER Study have now been analyzed for IgE ab to food allergens. Among 27 infants who presented with acute wheezing prior to age two, only one had a positive food RAST test (Fig.2). This child was 22 months old, was RAST positive to egg, and also had IgE ab to dust mite. None of the control patients in this group had IgE ab to the food allergens. Most of the wheezing patients in this group presented to the emergency room with their initial attack of wheezing and none had atopic dermatitis. We have compared the results for ER patients to infants referred for wheezing to the pediatric allergy clinic. The clinic patients differ in that they usually have had repeated episodes of wheezing and a few also have eczema. Similar to wheezing patients from the ER, the clinic patients have negative skin test responses to aeroallergens; however, 31% of 42 patients (who did hot have AD) had prick test responses to food allergens (predominantly to milk, egg, and soy). We believe that the reason for the low prevalence of IgE ab to food allergens by RAST among patients presenting to the ER reflects a low rate of atopy in this non-selected group of children. An alternative explanation is that the RAST tests for IgE ab to foods in these young children are less sensitive than skin tests for judging sensitization. However, the concordance between our RAST and skin tests for egg, milk, soy, and peanut is very high (86% overall). We plan to continue to evaluate the relationship between these methods for determining sensitization in infants from our clinic as well as in infants enrolled into the Prospective Study.

Among wheezing children ages 2 to 4, sera from 24% were food RAST positive. None had active eczema, but 77% had a history of prior wheezing. In these patients, the RAST tests for foods were positive in approximately the same percentage of sera as tests for inhalants (24% and 29%, respectively)(Table 2). In addition, 1/3 of the RAST positive children were identified by food tests alone. Thus, combining tests for IgE ab to common food and inhalant allergens in this age group is likely to enhance efforts to identify wheezing patients who are atopic and, therefore, candidates for allergen avoidance measures.

After age 2, the increase in IgE ab to foods in wheezing children was also evident in the results for each of the food allergens. After age 4, however, a marked increase in the prevalence of IgE ab to inhaled allergens (72%) was observed, whereas the percentage of patients with positive food RASTs in this group did not differ significantly from the percentage of patients with a positive food test ages 2 to 4 (Fig. 1A and 2). In addition, the majority of RAST positive wheezing patients (85%) over 4 were identified by tests for inhalants. Further analysis of the food data revealed that the prevalence of sensitization and the titers of IgE ab to food allergens in RAST positive wheezing patients after age 2 did not differ significantly from controls. However, these values were significantly lower than the IgE ab responses to foods in sera from AD patients. (Fig 3). Based on these findings, it is doubtful that routine tests for IgE ab to foods in children after age 4 would be clinically productive. Instead, these data support recommendations from others that food evaluations in older children should

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probably be reserved for wheezing patients with AD, or a history of food related symptoms (44-46).

(3) Total IgE levels and a family history for allergy:

In keeping with the low prevalence of IgE ab to food and inhalant allergens under age 2, the geometric means of total serum IgE levels in both wheezing and control children from the ER were low (Table I). Total IgE levels then rose rapidly in wheezing patients after age 2, and RAST positive wheezing patients had significantly higher total IgE levels than the RAST negative patients (GM = 365 and 67 IU/ml, respectively, $p < 0.001$). Prior to age 2, family histories for allergy-related symptoms (asthma, rhinitis, and eczema) were positive in a number of patients, but did not differ significantly between wheezing patients and controls. A higher prevalence of allergy in immediate family members (parents and siblings) was apparent in wheezing patients compared to controls after age 2 ($p < 0.001$), (Table 1). However, the prevalence of positive family histories did not differ significantly between RAST positive and RAST negative wheezing patients. In obtaining family histories in the ER, however, it was apparent to us that it was not always clear to many parents whether they wheezed or had allergies as children. Many of these families came from low income backgrounds. They frequently lack continuity in their health care and have never seen an allergist. In addition, many parents who were active smokers reported problems with coughing and had sinus complaints.

b) Analysis of mediators and eosinophils in nasal secretions and blood:

Eosinophils are often observed in nasal secretions of allergic individuals following natural allergen exposure. Their role in the late phase events of immediate hypersensitivity has been studied extensively (47). For this reason, we examined nasal smears from patients enrolled in the ER Study. Using the mean values recorded by three examiners, nasal smears containing greater than 10% eosinophils/100 cells counted were regarded as positive and judged to reflect at least moderate eosinophilia by reference to a scale proposed by Lim (48). In nasal smears from 21 wheezing patients under age 2, only one had a positive smear (a 22 month old child with IgE ab to mite). After age 2, the percentage of wheezing patients with nasal eosinophilia increased significantly and paralleled the increasing prevalence of IgE ab to inhaled allergens (Fig. 4). In addition, 91% of wheezing patients with positive smears were also RAST positive. More recently, we have begun to assess total eosinophil counts in blood. Thus far, the geometric means (GM) of counts in wheezing and control patients under age 2 is similar, whereas the GM for wheezing children ages 2 to 4 and over age 4 is significantly higher than controls (Fig 5).

We have recently enrolled eight patients from the ER in order to measure eosinophil cationic protein (ECP), tryptase, and kinins in nasal washes. The results indicate that these mediators can be measured and we have observed very high levels of ECP in washes from three wheezing patients over age 2 (Table 3). Recently, increased levels of ECP were reported in nasal secretions from RSV infected infants with bronchiolitis with the implication that virus-specific IgE ab may have been the stimulus for eosinophil degranulation (19). Because we have not observed nasal eosinophilia in RSV infected infants in our study, we shall be interested in the magnitude of ECP levels detected in these infants compared to controls. In previous studies, elevated kinin levels have been demonstrated in experimentally induced and natural rhinovirus infections (49). As potent vasoactive peptides, kinins have also been shown to induce symptoms of rhinitis and sore throat following nasal provocation (50) Among the patients analyzed, the highest kinin level measured was in an RSV infected infant with bronchiolitis (Table 3). Kinins have not previously been analyzed in infants with RSV and could

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potentially have a role as inflammatory mediators in the pathophysiology of wheezing in bronchiolitis.

c) Assessment of other risk factors for wheezing:

(1) Detection of virus in nasal secretions:

Virus was isolated in 70% of aspirates from wheezing patients less than age 2 (Fig 6). Of these 10 were positive for RSV, both by viral culture and by RSV antigen detection. Virus was also isolated from 22 of 70 (31%) aspirates obtained from wheezing children $\geq$ age 2 years. Most (73%) were positive for rhinovirus and three for RSV. Among this virus positive group over age two (64%) were RAST positive. This combination was not observed in any controls who had significantly fewer washes positive for virus ($p < 0.05$). The virus data from wheezing patients under age 2 support the importance of RSV as a major respiratory pathogen for wheezing in infancy. In wheezing children ages 2 to 4, however, RSV was isolated from only one patient and rhinovirus was the dominant virus cultured.

(2) Assessment of exposure to tobacco smoke:

Infants exposed to tobacco smoke in the home are reported to be at increased risk for more frequent respiratory tract infections and increased levels of airway responsiveness (9). Tobacco smoke exposure has also been reported to aggravate symptoms of asthma in older children, particularly in those exposed to mothers who smoke (23). In the ER Study, rates of exposure to one or more smokers at home, as judged by questionnaire analysis, were high (75% for wheezing patients and 58% for controls).

In addition to questionnaires, we examined the utility of measuring levels of cotinine, a nicotine metabolite, in saliva from patients in the ER Study. Cotinine was measured by a competitive inhibition RIA and results have been shown to correlate well with measurements in blood and urine (22,51). A striking observation was that 14 of 19 (74%) of wheezing infants less than age 2, who were exposed to smoke at home, had cotinine levels $\geq$ 10 ng/ml, a level reported to be suggestive of heavy passive smoke exposure (22) (Fig 7). The geometric mean of cotinine determinations was significantly higher for wheezing patients under 2 than for wheezing patients over 2 (9.8 and 3.6 ng/ml, respectively, $p < 0.05$). We speculate that the higher levels for smoke exposed children under 2 reflects increased exposure due to greater dependence on and proximity to their parents. Although cotinine has also been detected in the breast milk of lactating mothers who smoke, no infants, by questionnaire analysis, were being breast fed when they were enrolled in the ER Study. Among control patients under age two, few were exposed to tobacco smoke. After age two, however, cotinine concentrations in saliva from wheezing and control patients did not differ significantly.

For the last 72 patients enrolled (35 wheezing children and 37 controls), we began to examine exposure to smoke from mothers, fathers, and others living in the home. Of this group, 60% of wheezing patients and 75% of controls were exposed to smoke from mothers. Under age two, histories for maternal smoke exposure were not sufficient for statistical analysis. Over age 2, higher cotinine levels were detected in those exposed to smoking mothers, although the levels detected did not differ significantly between wheezing patients and controls. As these studies continue, we expect that salivary cotinine levels in infants (both wheezing and controls), who are exposed to smoke from mothers will be a useful marker for heavy smoke exposure and be high enough to monitor periodically in infants enrolled in our Prospective Study. In addition, cotinine levels in infants appear to be high enough to

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be used in future studies to evaluate strategies to alter smoke exposure at home.

Conclusions and implications:

In the ER Study, emphasis given to the assessments of IgE ab to allergens, viral infection, and tobacco smoke exposure have proven to be very useful in characterizing risk factors for wheezing in children with respect to their age. Objective measurements of these three major inducers of airway hyperreactivity have not been examined in the same patients before. Results from this study indicate that when wheezing infants present in a non-selected fashion to an emergency room, they are likely to have a high rate of viral respiratory tract infection, particularly RSV, and a low rate of sensitization to inhalant and food allergens. Nasal and peripheral blood eosinophilia are also not a common finding in these patients. These data indicate that wheezing in infancy may not be selective for individuals who are atopic and may not be selective for those predisposed to develop asthma.

After age 2, IgE ab to inhalant allergens in wheezing patients became increasingly frequent and was significantly more common than in controls after age 4. For wheezing children ages 2 to 4, however, tests for IgE ab to foods were positive in approximately the same percentage of patients as tests for inhalants, and 1/3 of RAST positive children were identified as atopic by food tests alone. These children also had other findings characteristic of asthma including nasal and peripheral blood eosinophilia and elevated total IgE levels. In addition, most viral pathogens identified in this age group were rhinovirus. After age 4, the majority of wheezing children with serum IgE ab were identified by tests for inhalants, whereas the prevalence and titers of IgE ab to individual food allergens in these children were not significantly different from controls. Most RAST positive wheezing patients in this age group also had nasal eosinophilia.

A striking observation in the ER Study was that 66% of wheezing patients had evidence for two or more of the risk factors examined compared to 28% of control patients, $p < 0.001$. Passive smoke exposure was common among wheezing children at all ages. In the ER however, high salivary cotinine levels ($\geq$ 10 ng/ml) suggesting heavy smoke exposure were significantly more common among the children under age 2. Odds ratio analysis revealed that children less than age two in this study were 8 times more likely to be wheezing if they had a positive viral culture. They were also more likely to be wheezing if they were smoke exposed and had cotinine levels $\geq$ 10 ng/ml. If they had an elevated cotinine level and a positive virus culture the odds for wheezing were the same as having an elevated cotinine level alone.After age 2, RAST positivity was strongly associated with wheezing, but significantly more so if patients were also infected with virus. (Table 4). Implicit in these findings is that proper management of wheezing in these patients should include efforts to identify environmental risk factor which can be modified (e.g. indoor allergen and tobacco smoke exposure). Currently, the management of wheezing in the ER usually focuses exclusively on medications, after which patients are sent home to unaltered environments.

2) Atopic Dermatitis (AD) Study:

It is becoming increasingly apparent that immediate hypersensitivity to inhalant allergens is very common among patients with AD (52,53). This finding is consistent with the increased risk which children with AD have for developing allergic respiratory symptoms (allergic rhinitis and asthma). In a cross-sectional study of AD, we evaluated 58 patients with moderate to severe eczema in the Dermatology Clinic at the University of Virginia using criteria established by Hanifin and Rajke (54).

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Under age 2, 38% of AD patients were sensitized to inhaled allergens by RAST (Fig. 8). All but one of these sensitized infants had IgE ab to indoor allergens (dust mite and cat), and 3 children who were sensitized to these allergens were less than one year of age.

After age 2, a striking increase in the prevalence AD patients with positive RASTs for inhalants was observed (61% of children ages 2 to 4, and 82% of patients over age 4)(Fig. 8). Again, most RAST positive children had IgE ab to dust mite and/or cat allergen. These data suggest that children with AD can develop aeroallergen sensitization prior to age two and, therefore, allergy evaluations to assess IgE ab to inhalant (and not only food) allergens during infancy should be recommended. In addition, the results indicate a need for controlled studies to determine the effects of indoor aeroallergen avoidance on the timing of sensitization and development of allergic respiratory tract symptoms in these patients.

3) Human IgE ab Responses to Purified Dust Mite Allergens:

We have previously reported the purification of *Dermatophagoides* dust mite allergens *Der p* I and *Der f* I, from *D. pteronyssinus* and *D. farinae*, respectively (55,56). These allergens are referred to as Group I mite allergens and the majority of pediatric and adult patients with mite allergy (78-80%) have been shown to have IgE ab to these antigens (56). More recently, we purified *Der f* II and *Der p* II (the Group II mite allergens) using monoclonal antibody (mAb) affinity chromatography (57). These allergens (MW 15 kd) show 88% amino acid sequence homology and, correspondingly, show extensive cross-reactivity with human IgE and murine IgG antibody. We have also purified a third allergen from *D. farinae*, *Der f* III, which is structurally and immunochemically distinct from the Group I and Group II *D. farinae* allergens. Together, these allergens have been valuable for ongoing analyses of IgE as well as IgG ab responses in patient's sera.

In direct antigen binding experiments, IgE ab to highly purified preparations of radiolabelled *Der f* I and *Der f* II were detected in 80% and 92% of sera, respectively, obtained from 98 children judged to be mite allergic by RAST. Linear regression analysis of IgE ab to these proteins showed an excellent correlation between IgE ab to *Der f* I by RIA and to a *D. farinae* crude extract by RAST ($r=0.68$, $p < 0.001$) and between IgE ab to *Der f* II and *D. farinae* (($r=0.81$, $p < 0.001$), further suggesting that both *Der f* I and *Der f* II are major allergens. More recently, Calkhoven et al studied 18 young children (14 had a history of atopic dermatitis) who developed IgE ab to mite allergen at a mean age of 32 months (range 11 to 60 months) and reported that 15 of these children produced IgE responses to allergen components distinct from *Der p* I and *Der p* II (58). We have not yet examined IgE ab to Group I and Group II allergens in very young children and plan to do so in patients enrolled in the Prospective Study.

We have also raised monoclonal antibodies (mAb) specific for the Group I and Group II allergens (59). When coupled to CNBr activated RAST discs, or activated microtiter plates, these antibodies can be used to capture mite allergens from crude extracts and, in turn, can be used to test sera for allergen specific IgE ab. Using this method, serum IgE ab to *Der f* III was detected in 64% of 44 mite allergic patients who had either asthma and/or AD. By linear regression analysis, however, the correlation between levels of IgE ab to *Der f* III and IgE ab to *D. farinae* by RAST was not strong ($r=0.24$). Thus, while the majority of patients studied were sensitized to *Der f* III, *Der f* I and *Der f* II are likely to be immunodominant and IgE responses to these allergens will be monitored in our studies.

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The mAbs to mite allergens have also been used to develop assays for measuring Group I and Group II allergens (60,61). The mAb assays for mite allergen concentrations in dust samples correlate well with mite body counts, RAST inhibition analyses, and with results using an inhibition RIA for measuring Group I allergens (59). The mAb assays for Group I allergens are species specific for either *Der f* I or *Der p* I. Their results are normally added together in analyzing dust samples from homes where both *D. farinae* and *D. pteronyssinus* species co-exist. These assays are also useful in epidemiologic studies which require the species identification of dust mites. The mAbs used in the two-site assay for Group II allergens bind strongly to both *Der f* II and *Der p* II and have greater usefulness in monitoring mite allergen exposure irrespective of which *Dermatophagoides* species is present. In the Prospective Study, these assays will be used to measure mite allergen concentrations in dust samples from patients' homes and results will be analyzed with respect to the presence of IgE ab to these allergens in patients' sera. Similar assays developed in our division by Dr. Martin Chapman to measure cat (*Fel d* I) and cockroach (*Bla g* I and *Bla g* II) allergens in dust samples will also be used in these studies (see support letter, Appendix B).

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Table 1. Patient Characteristics:

	CHILDREN <2		CHILDREN ≥2	
	Wheezing Patients	Controls	Wheezing Patients	Controls
Number of patients	27	13	70	53
Mean age (yr)	0.9	1.2	7.0	9.9
Age range	0.3-1.8	0.5-1.8	2.0-15.4	2.3-16.1
Male (%)	56	27	66	64
Race: Black (%)	67	45	51	33
Family history (%)*	56	82	83	47
Total IgE‡	15	4	166	27
(95% C.I.)	(9-26)	(2-10)	(110-251)	(18-42)
Payment Requirements:§				
100%	15	58	18	31
10-75%	11	0	26	13
0%	73	42	56	56

‡ The total IgE levels in sera are expressed as geometric means in IU/ml as are 95% percent confidence intervals. § Payment requirement categories reflect the proportion of the medical bill charged to the patient's family based on annual family income. The percentages of families required to pay 100%, 10-75%, or 0% of their child's bill are shown. Sera from 97 wheezing and 55 control patients were available for measurements of IgE ab to both inhalant and food allergens.

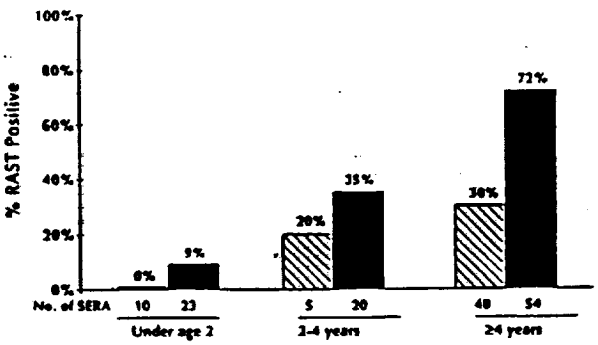

Fig. 1A: Percentage of patients with IgE ab to inhaled allergens. Wheezing patients (black bars). Controls (hatched bars).

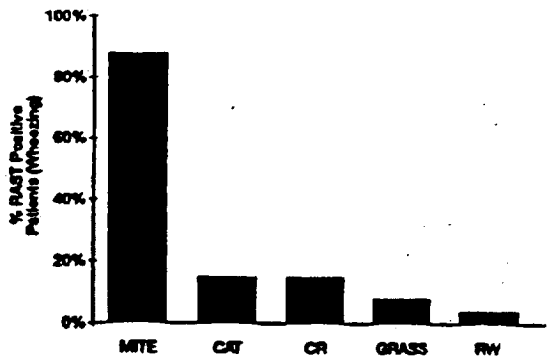

Fig 1B: Percentage of all RAST positive wheezing patients with IgE ab to mite, cat, cockroach (CR), grass, and ragweed (RW) extracts.

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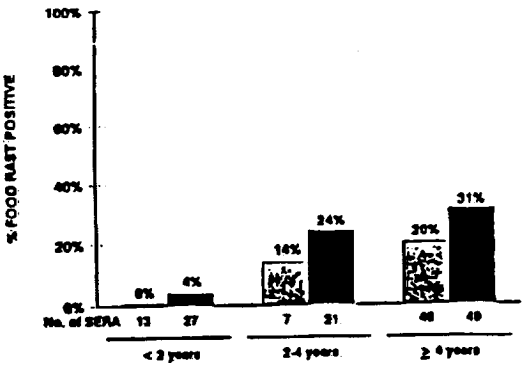

Fig. 2: Percentage of patients with IgE ab to food allergens. Wheezing patients (black bars). Controls (hatched bars).

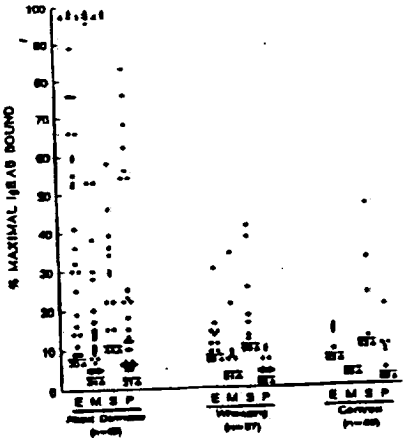

Fig. 3: Titers of IgE ab to egg (E), milk (M), soy (S), and peanut (P) in children ≥ 2yrs.

TABLE 2: Positive RAST Responses to Food and Inhalant Allergens in Wheezing Children*

Sera with IgE ab to one or more:	Age < 2 y (n = 27)		Age 2 - 4 (n = 21)		Age ≥ 4 (n = 49)	
	Positive‡ Patients (%)	Total§ IgE	Positive‡ Patients (%)	Total§ IgE	Positive‡ Patients (%)	Total§ IgE
Food Allergens¶	1(4)%	59	5(24)%	140	15(31)%	217
Inhalant allergens•	2(8)%	102	6(29)%	155	29(59)%	338
Foods only	0(0)%	NA	3(14)%	137	5(10)%	55
Inhalants only	1(4)%	178	4(19)%	160	19(39)%	225
Foods and Inhalants¤	2(8)%	102	9(43)%	149	34(69)%	259

Sera from 97 wheezing and 55 control patients were available for measurements of IgE ab to both inhalant and food allergens. ‡ Number of RAST positive patients are followed by the % who were positive in parentheses. § Total serum IgE levels are reported as geometric means in IU/ml.

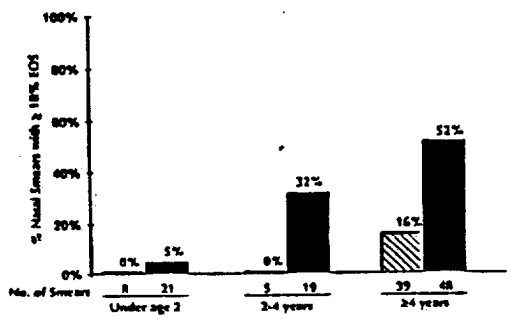

Fig. 4: Percentage of nasal smears containing≥ 10 eosinophils/100 cells counted. Wheezing patients (black bars). Controls (hatched bars).

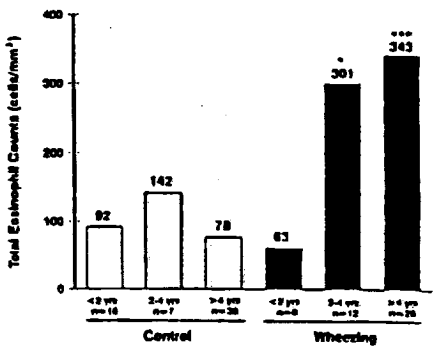

Fig. 5: Mean (geometric) total blood eosinophil counts. Wheezing patients (black bars). Controls (open bars).

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TABLE 3: Analysis of mediators in ER Patients

Wheezing Patients	RAST Inhalant	Blood Eos (mm³)	Nasal Wash Eos	ECP	Tryptase	Kinins
4 mos.	NEG	87	0	68	21.6	13,790*
5 mos.	NEG	225	0	26	<8.6	300
2½ yrs.	–	2295	2+	>200	62.1	1725
10 yrs.	POS/MITE	262	3+	>200	14.6	703
Control Patients						
4 mos.	–	60	0	55	<8.6	1650
2½ yrs.	NEG	190	0	46	9.0	389
16 yrs.	NEG	184	0	<2	<8.6	260
11 yrs.	NEG	67	0	9	<8.6	218

*Indicates a nasal wash positive for RSV antigens. Total blood eosinophil counts are reported as cells/mm³. ECP, tryptase, and kinin values are in microgram, manogram, and picogram quantities, respectively.

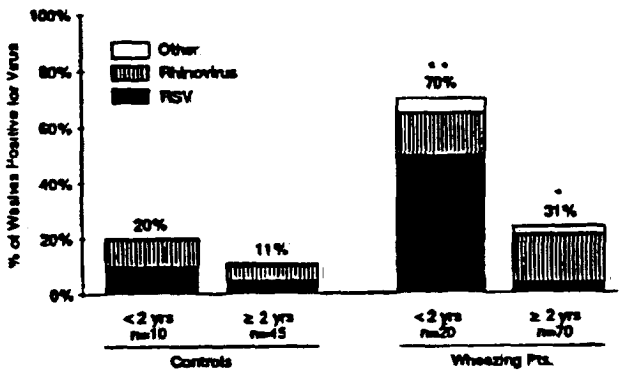

Fig. 6: Viral detection in nasal aspirates.

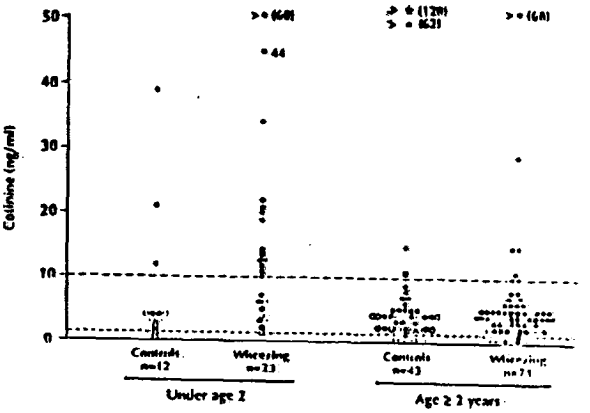

Fig. 7: Salivary cotinine. Solid circles are patients who were exposed and open circles are patients who were not exposed to tobacco smoke by questionnaire.

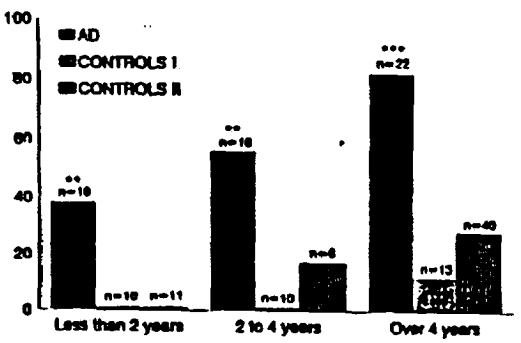

Fig. 8: Percentage of AD patients with IgE ab to inhaled allergens. Control groups I and II come from the pediatric clinic and ER, respectively.

Table 4. Odds Ratios for Wheezing Among Children with a Positive RAST, Virus Culture, or Elevated Cotinine Level

Risk Factors	Odds Ratio* Under age 2	Over age 2
RAST	†	4.5 (2.0-10.2)***
VIRUS	8.2 (1.3-51.0)*	3.7 (1.3-10.6)*
COTININE ≥10ng/ml	4.7 (1.0-21.3)*	0.6 (0.2-2.2)
RAST and VIRUS	†	10.8 (1.9-59.0)***

* Reported odds ratios are univariate analyses followed in parentheses by 95% confidence intervals. † Not determined because of insufficient positive patients.

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9. EXPERIMENTAL PLAN

In these investigations, we plan to evaluate patients using cross-sectional, case control and observational cohort study designs. The case control (ER) study will provide information regarding the prevalence of data (e.g. IgE ab to inhaled and food allergens) in patients with respect to age. The observational cohort (Prospective Study) has the advantage of following individual patients over time to determine the incidence of sensitization to inhaled and food allergens in patients and the proportion of infants with these disorders who will continue to experience wheezing episodes after age two.

1) *Specific Aim 1:*

Emergency Room (ER) Study.

Thus far, we have enrolled 178 patients into the ER Study. The data analyzed are presented in Section 3 (Preliminary Studies). Over the next 18 months, we plan to enroll 100 wheezing patients (30 patients < age 2, 30 patients ages 2 to 4, and 40 patients ≥ age 4) and 80 control patients (20 patients < age 2, 20 patients ages 2 to 4, and 40 patients ≥ age 4). Based on our data thus far, 9% of wheezing patients under age 2 and 72% of patients over age 4 had IgE ab to one or more inhaled allergens. Thus, power calculations indicate that, by enrolling 30 wheezing subjects younger than age 2 and 40 wheezing children over age 4, we will have at least a 95% chance of showing a significant difference between these two groups with respect to their rates of sensitization to aeroallergens based on two-sided hypothesis tests conducted at the 0.05 level of significance (62). In addition, based on the prevalence of sensitization to inhaled allergens of 30% for controls over age 4, we should have at least a 95% chance of demonstrating a significant difference between the proportion of wheezing and control children ≥ age 4 who have IgE ab to inhaled allergens by enrolling 40 patients in each group.

We will continue to enroll wheezing patients (ages 2 months to 16 years) in a non-selected manner based on the diagnosis of bronchiolitis or asthma requiring treatment (e.g. usually an inhaled beta-agonist). In keeping with the definition of asthma proposed by the American Thoracic Society (63), asthma will be suspected in children who give a history of recurrent episodes of wheezing, periodic cough, chest tightness, or dyspnea with associated changes in severity which may occur spontaneously, or in response to therapy. (A number of young children who are presenting with their first attack of wheezing are likely to be seen in the ER , however, and some patients with status asthmaticus may respond poorly to asthma medications). Bronchiolitis will be suspected in children who present with wheezing prior to age 2 who are treated in the ER with at least a bronchodilator. The following patients will be excluded from the study: 1) infants suspected of having bronchopulmonary dysplasia by virtue of requiring oxygen and ventilatory support at birth; 2) patients suspected to have a foreign body or congenital abnormalities of the cardiovascular or pulmonary system; 3) patients with cystic fibrosis; and 4) patients who have been given systemic steroids within a week of their ER visit which may interfere with our analyses of eosinophils, ECP, and tryptase. Other than steroids, medications used to treat asthma should have a minimal effect on this study. A few patients with more severe bronchospasm (thus far, only 2 patients omitted from our study) may receive epinephrine subcutaneously which can demarginate eosinophils, elevate their numbers in the peripheral circulation, and obviate analysis of total blood eosinophil counts. Control patients (ages 2 months to 15 years) will be enrolled if they present with no respiratory tract symptoms and no history of asthma or atopic dermatitis.

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Enrolling patients with respect to time of year:

From January through December 31, 1991, 394 children (birth to 15 years) presented to the ER for wheezing. Of these, 104 patients were less than age 2 (71 were diagnosed with bronchiolitis and 33 with asthma) and 290 patients were over age 2 (17 diagnosed with bronchiolitis and 273 with asthma) (Fig. A, below). From January 1 through December 31, 1992, 489 wheezing children presented to the ER (173 patients were < age 2 and 316 were over age 2). The seasonal variation in visits for these children (both under and over the age of 2) was very similar to the pattern observed for 1991.

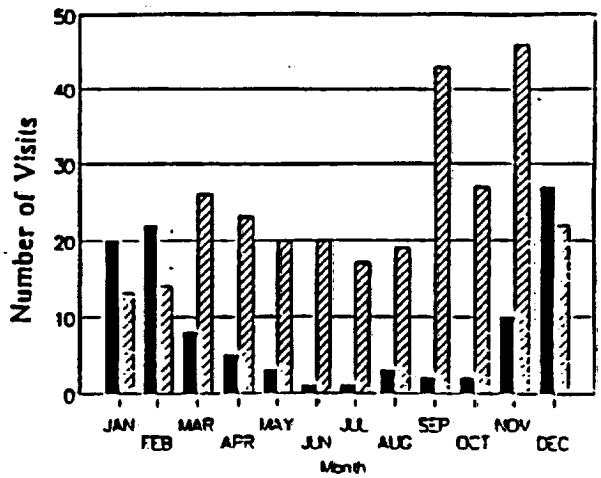

Fig. A: Emergency Room Visits for Wheezing, Black bars = Children 0-2 years, Hatched bars = Children > 2 to 15 years.

[These figures are based on ICD-9-CM codes for bronchiolitis and asthma and do not include an additional 112 patients who were identified by the ICD-9-CM code for "Other Lung Diseases", a number of whom were given the diagnosis of reactive airways disease.]

The majority of children under age 2 presented to the ER when the prevalence of RSV infection was high. Older asthmatics were seen more often in the Spring and Fall. The time of year when a patient presents to the ER with wheezing is expected to influence the type of virus isolated from nasal secretions. However, the time of year should not significantly influence titers of allergen specific IgE ab in serum for either wheezing patients or controls, aside from modest increases in levels during periods of increased allergen exposure. Similarly, with persistent smoking in the home, salivary cotinine levels are not likely to change much except during summer months when older children spend more time outdoors (64). Thus far, we have been enrolling very few patients during summer months since wheezing patients present less frequently with symptoms at that time of year. As we continue this study we shall extend the enrollment of patients into the summer.

Method of enrollment:

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The ER Study has been approved by the Human Investigation Committee at the University of Virginia (see consent form "Risk Factors for Acute Episodes of Wheezing in Infants and Children", Appendix A). Informed IRB will be obtained from the parent (and child, if old enough), after describing the purpose of the study and procedures. All patients will be enrolled by physicians (fellows in training and faculty) from the Allergy Division at the University of Virginia. A questionnaire focused on birth history (to exclude patients who received oxygen or ventilation support as risk factors for bronchopulmonary dysplasia); breast feeding; prior history of wheezing and symptoms related to allergy; family history for allergy, asthma, and eczema; enrollment in day care; levels of parent education; and smoking in the home will be administered to a parent, (and child, if old enough), (Appendix A). A second questionnaire documenting the patient's signs and symptoms, a Wood's score assessment, treatment given in the ER, presence or absence of eczema, and requirement for hospitalization will be completed by the physician enrolling the patient (Appendix A). Emergency room records will also be available for each patient as will information regarding the annual income status of parents which is obtained when patients register in the ER.

Samples collected from each patient will include:

a) Blood (4-5 ml) obtained by venipuncture. Of this, 3 to 4 ml will be centrifuged and serum stored at -70° C for analysis of total IgE, IgE ab to inhaled and food allergens, and ECP. One-half to 1 ml will be used for a CBC and differential cell count to assess peripheral eosinophilia.

b) saliva (0.5 to 1.0 ml) for cotinine analysis. Prior to age 4, saliva will be obtained from the floor of the mouth using an ear suction syringe. After age 4, most children can spit into a plastic collection cup. Following centrifugation to remove mucin, supernatants will be stored at -20° C. Saliva samples will also be obtained from parents (most often the mother) who accompany their children.

c) nasal secretions. For children less than age 5, approximately 2 ml of normal saline will be instilled into each nares and secretions aspirated from the nasopharynx using a flexible suction catheter. The catheter will then be rinsed with an additional 1 ml of saline. The yield from this procedure is approximately 3 ml of fluid including mucoid secretions (often more is obtained). Approximately 2.5 ml of saline will be instilled into the nares of older patients as they hold their breath, after which they will blow their secretions into a sterile plastic cup. For virus detection, 0.5 ml will be transferred to vials containing one ml of veal infusion broth (pH 7.4) treated with vancomycin (20 μg/ml), gentamicin (50 μg/ml) and amphotericin B (1 μg/ml) and stored at 4° C. Using a cotton swab, mucoid secretions will also be transferred to a glass slide and allowed to dry for analysis of eosinophils. The remaining secretions will be centrifuged and the supernatant stored at -70° C for analysis of tryptase, kinins, ECP, and anti-RSV IgE ab in keeping with methods previously described (17,65-67).

a) Measurements of IgE antibody:

(1) Serum IgE ab to inhalant and food allergens:

Serum IgE ab will be measured by RAST using CNBr-activated cellulose discs coated with allergen (18). Extracts obtained from Hollister-Stier Laboratories, (Spokane WA), will include *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, cat epithelium, short ragweed pollen,

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rye grass pollen, and mixed cockroach extracts with approximately 0.1 to 0.5 μg of major allergen (e.g. *Der f* I, *Fel d* I, *Amb a* I, or *Lol p* I) coated per disc. A micro-RAST procedure will be used to conserve pediatric sera, as previously described (41). Using horse serum as a diluent to minimize non-specific binding, sera will be tested at dilutions of 1/4 and 1/10. Five positive and six negative control sera will be run in parallel with each assay. In addition, sera with high total IgE levels will also be diluted further to obtain a level < 1000 IU/ml. Control sera containing high titers of IgE (≥ 1000 IU/ml), but without IgE ab to the allergen tested will be included in each assay. Results will be reported in RAST units/ml determined from a reference control curve. Sera containing ≥ 40 RAST units/ml to an allergen (equivalent to ~ 4ng/ml of allergen specific IgE ab) will be considered positive (2).

For sensitization to food allergens, sera will be screened for IgE ab to egg, milk, soy, and peanut allergens. From the last 63 patients seen in our clinic who had positive food skin tests, and were seen for asthma and/or AD, 92% had responses (≥ 3x3 mm larger than a saline control) to at least one of these allergens. The starting materials for preparing egg and milk allergen extracts include crude dried chicken egg white (Sigma # A5253) and beta-lactoglobulin A and B (Sigma #L2506). To test for IgE ab to soy and peanut allergens, extracts of isolated soy protein (Ralston Purina Co., St. Louis, MO, lot # CGC-E0070) and a defatted Florunner peanut preparation have been kindly provided by Dr. Wesley Burks, Little Rock, AR. For each food allergen, sera from 6 to 8 patients seen in clinic for asthma and/or AD and who had high titers of serum IgE ab to the allergen tested have been pooled to establish a control curve. Positive and negative sera will be included as controls in each assay as described above.

(2) IgE ab to dust mite allergens:

Using methods described in section 3, we plan to measure serum IgE ab to the *D. farinae* mite allergens *Der f* I and *Der f* II using a monoclonal ab (mAb) modified RAST assay. Briefly, allergen specific mAb will be coupled to CNBr-activated cellulose discs and then incubated with a crude mite extract obtained from Hollister-Stier, Spokane, WA to capture mite allergen. Radiolabelled goat anti-human IgE ab will be added to detect bound IgE from patients' sera. This assay uses 17 μl of patient's serum per test (using dilutions of 1/4 and 1/16) and has the advantage of not requiring highly purified allergen preparations needed for antigen binding RIAs (41)

(3) Total serum IgE levels:

Measurements of total IgE in sera will be done with a two-site immunoassay using anti-Fc-epsilon monoclonal antibodies (kindly provided by Dr. Andrew Saxon, UCLA) on solid phase together with affinity purified goat anti-human ab as the detecting ab (2). The sensitivity of this assay is approximately 1 ng of IgE/ml.

b) Analysis of mediators and eosinophils in nasal secretions and blood:

(1) For mediator analysis, 0.4 ml of nasal wash fluid will be added to 0.1 ml, 0.2M EDTA (pH 6.5 to 7.5) for kinin determinations in the laboratory of Dr. David Proud (see consultant letter). The assay for kinins is a competitive inhibition RIA which uses a polyclonal rabbit antiserum to bradykinin and radiolabelled Tyr-bradykinin (66). The assay will also detect other kinin products, lysylbradykinin and methionyllysylbradykinin. No other cross-reacting materials are known. Studies by Proud and collaborators have shown elevations in kinin levels along with increased levels of histamine after nasal challenge with allergen in allergic individuals (66). During experimental

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rhinovirus infections, however, kinin levels increase markedly, whereas histamine concentrations do not (67). These results have been confirmed in nasal secretions from adults during natural rhinovirus colds (49). No studies yet have assessed kinins in secretions from RSV infected individuals, or from infants with bronchiolitis and other virus infections. An additional 0.5 ml of nasal wash will be used to assess tryptase in Dr. Proud's laboratory (65). We shall also save 0.5 ml of wash fluid for ECP analysis using a competitive inhibition fluorometric (CAP) assay for ECP, Pharmacia Diagnostics, Uppsala, Sweden.

(2) To study nasal eosinophils, glass slides with dried secretions will be treated with Hansel stain (Lide Laboratories, Florissant, MD). Each slide will be examined under oil immersion by two individuals blinded to the identity of the patient. Using mean eosinophil values recorded by the two examiners, smears containing greater than 10% eosinophils/100 cells counted are regarded as positive and judged to reflect at least moderate eosinophilia by reference to a scale proposed by Lim (48). Total blood eosinophil counts will be obtained from blood samples sent to the University of Virginia Hospital clinical laboratory which uses an H*1 Blood Cell Analyzer (Technicon Instruments Corporation, Tarrytown, NY) to identify eosinophils based on size, gránularity and cytochemical staining with myeloperoxidase. This third-generation automated blood-cell counter provides data from counts of approximately 100 times as many cells as those counted using conventional microscopic techniques (68). Results will be correlated with measurements of ECP in serum using the assay from Pharmacia Diagnostics, Uppsala, Sweden.

(3) Thus far, we have not observed eosinophils in nasal secretions from any RSV infected infant with bronchiolitis. Others, however, have reported the detection of IgE ab specific for RSV antigen as well as increased levels of ECP in nasal secretions from RSV infected infants (17,19). Because of difficulties measuring IgE ab to RSV in serum from these same patients, the authors conclude that this response may occur predominantly at the mucosal surface (69). If functional, these antibodies should stimulate mediator release from mast cells in order to contribute to symptoms and one would also expect to see eosinophilia. Therefore, if we observe elevated levels of tryptase and ECP in nasal secretions from RSV infected infants then we shall also try to measure IgE ab to RSV and will obtain RSV antigen for these assays using methods previously described (70). The experience and methods for measuring antibodies in nasal secretions are available in our laboratory (71).

c) Assessment of other risk factors for wheezing:

(1) For viral analyses, 0.5 ml of nasal wash fluid and secretions will be transported to the Clinical Virology Laboratory at the University of Virginia and processed within four hours of collection using a commercially prepared direct fluorescent antibody assay to stain for RSV antigen (Ortho Diagnostic Systems, Inc., Raritan, NJ). To isolate other viruses, (e.g. rhinovirus, parainfluenza viruses, influenza A and B viruses, adenovirus, enterovirus, as well as RSV), each aspirate will be used to inoculate WI-38 or MRC-5 strain human embryonic fibroblasts, A549, primary rhesus monkey kidney, and HEp-2 cell lines as described in the consultant letter from Dr. Frederick Hayden. In addition, each sample will also be tested for rhinovirus RNA in Dr. Hayden's laboratory using a nested primer PCR assay. This assay is being investigated as a more sensitive method for detecting rhinovirus in nasal secretions than conventional culture techniques.

(2) For measurements of cotinine in saliva, we are using an inhibition RIA using the method described by Van Vunakis, et al (51). This RIA is sensitive for cotinine concentrations $\geq$ 1.0

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ng/ml, and we have purchased the reagents for this assay from Dr. Van Vunakis (Brandeis University). Mean salivary cotinine concentrations will be compared between wheezing and control patients and correlated with exposure to tobacco smoke from mothers, fathers, and the others living in the home. Self-reported smoking by older school-aged patients will also be recorded (see questionnaire, Appendix A). In most cases mothers and/or fathers are available in the ER and saliva samples will be obtained from them to test for cotinine. Correlations between cotinine levels in the saliva from patients and their parents will also be made.

d) Analysis of history and physical findings:

Each patient's history for allergic symptoms, family history for allergy, medications used, and exposure to tobacco smoke will be recorded (see Pediatric Wheezing Study Questionnaire, Appendix A). Physical findings will be documented (Pediatric Asthma ER Physical Assessment Form, Appendix A) and the patient's socioeconomic status assessed. The educational background of parents will be determined by questionnaire and the income status for each family will be obtained from the UVA Dept. of Medical Records which stores this information when patients are hospitalized or seen in the clinics or ER. Approval of this study by the IRB at the University of Virginia requires that this information and the patient's identity will remain confidential.

2) *Specific Aim 2:*

Prospective Allergy Study

In this study, we are enrolling groups of infants who present with wheezing between the age of 2 to 18 months. Infants with AD and healthy controls in the same age range will also be enrolled. These children will be followed periodically until age four for the development of IgE ab to food and inhalant allergens in relation to total IgE levels, nasal and peripheral blood eosinophilia, symptom and medication scores, and levels of allergen and tobacco smoke exposure at home. The immediate family history (parents and siblings) for allergic symptoms will also be ascertained. In addition, parents will be tested for sensitization to the same inhalant allergens used to test study patients. Half of the patients in this study will be enrolled during the first grant year and half during the second. The objectives of this investigation will be to: 1) establish diagnostic guidelines which can be used by clinicians to identify patients who are at risk for developing inhalant allergy and asthma, and 2) define in greater detail the evolution of immune responses to allergens in early childhood among groups of infants who will vary in their risk for developing reactive airway disease.

The development of IgE ab to inhalant allergens will be the primary outcome measure over the study period during which we will follow children for 3½ to 4 years. As described in the long range plans, we plan to follow children enrolled in this study for an additional 5 to 7 years. The purpose will be to examine the development or persistence of symptoms in subjects relative to their presenting diagnosis as infants and the timing of their sensitization to inhalant and food allergens.

Selection of Patients:

Group I (Wheezing infants): Children will be enrolled into 3 groups of infants who are likely to vary in their risks for recurrent wheezing:

Group IA: Thirty children will be enrolled from the pediatric ER. We have already enrolled 14 patients in this group and half of them have nasal washes positive for RSV. Based on data thus

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far, we don't expect these infants to have a high rate of atopy and speculate that the prevalence of IgE ab to inhalants by age 4 in this group is not likely to differ from controls. Consistent with our clinical experience, we speculate that 70-80% of these children will outgrow their problems with wheezing after their second or third year of life. This has not been clear, however, from previous prospective studies. The importance of examining this group is to determine whether bronchiolitis, in the absence of atopic risk factors, can predispose infants to subsequent wheezing and asthma.

Group IB: Thirty children who have been admitted to the hospital for wheezing will be enrolled. Because hospitalization alone is not the best predictor of disease severity, these patients will be enrolled only if their O_2 saturation by oximetry drops below 90% during the first day of admission which requires management in the Pediatric Intensive Care Unit at the University of Virginia. Although it is not clear that those with more severe symptomatology are more likely to have an atopic predisposition, IgE ab to RSV has been reported in infants hospitalized with bronchiolitis (17). It is also possible that these children have smaller airways and/or enhanced airway reactivity and are, therefore, more likely to wheeze with infections (11). Whether they also have an increased risk for recurrent wheezing during the subsequent 3 years will be examined in this study.

Group IC: Candidates for enrollment in this group are children referred to the pediatric allergy clinic who have 1) at least one physician documented episode of wheezing requiring treatment with a bronchodilator and 2) at least one parent with a positive history for allergic symptoms. In addition, parents will be skin tested as described below for sensitization to the same 5 inhaled allergens used to evaluate their child. Serum will also be obtained from parents for measurements of total IgE and serum IgE ab.

The first thirty children who have a parent with a positive skin test to one or more inhaled allergens will be enrolled in this group and one-half of these children will be enrolled if they also have a positive prick skin test for IgE ab to egg, milk, soy or peanut allergen. Infants who have AD will not be enrolled. This subgroup will contain children with a strong background for atopy and will allow us to examine whether infants who are sensitized to food allergen and have an allergic parent have a greater risk for developing IgE ab to aeroallergens and respiratory symptoms than those with an atopic parent alone. We are assuming that the children whose parents are skin test negative and who are not enrolled will not differ significantly in their risks for developing sensitization and symptoms from patients in Group IA. To test this we will contact 20 of these patients at age 4 and arrange a clinic visit for evaluation.

Group II (Atopic Dermatitis infants): Using criteria proposed by Hanifin and Rajke (54), we plan to enroll 30 infants with moderate to severe AD and who require topical steroids (daily, or intermittently) to control skin rash. These patients will be recruited from the Immunodermatology Clinic at the University of Virginia (see support letter from Dr. Barbara Wilson, Appendix B). Based on our cross-sectional analysis of children with AD, most of these infants will have IgE ab to food allergens at the time of enrollment, 30-40% of them are likely to develop IgE ab to aeroallergens (predominantly indoor allergens) by age 2, and $\geq$ 80% are likely to develop sensitization to inhaled allergens by age 4. Eight patients have been enrolled in this group thus far.

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Group III (Asymptomatic control infants): These children will be enrolled into 2 groups, one of which will include children with an atopic background:

Group III A: Thirty patients, who have no personal history for wheezing or AD, will be enrolled from the General Pediatric Clinic (see letter of support from Dr. Gregory Hayden, Appendix B). The socioeconomic background of patients attending this clinic is similar to those who utilize the pediatric ER, and will be evaluated in this study. In keeping with estimates of inhalant allergy in the general population we speculate that 20-30% of this group will develop IgE ab to aeroallergens by age 4. (This is consistent with results from our ER Study). If it is true that bronchiolitis in infancy is not selective for atopic children, then we would also predict a similar rate of sensitization to inhalants in these children (i.e. from Group IA). By contrast, we expect that at least 80% of infants with AD will develop IgE ab to inhalants by age 4. Based on these rates of sensitization (80% for AD patients and 30% for control or ER wheezing patients, we would have at least 95% power to detect a significant difference between the AD and control patients or between AD and wheezing patients in Group IA if we enrolled 23 infants into each group (62). Seven children have been enrolled in this control group.

Group III B: Thirty children will be enrolled from the pediatric allergy and general pediatric clinics who have no personal history for wheezing or AD. Analogous to wheezing patients in Group IC, however, all children in this subgroup will be enrolled if they have at least one allergic parent. Similar to patients in Group IC, skin tests will be done to document sensitization in the parents and one-half of these children will be enrolled if they have a positive skin test to egg, milk, soy, or peanut allergen. Patients in this group differ from those in Group IC only because they have not experienced a wheezing episode and are included for comparison to determine whether those with wheezing in infancy together with an atopic background are more likely to develop asthma.

Method of enrollment:

Approval by the Human Investigation Committee (IRB) at the University of Virginia for the Prospective Study has been obtained (see IRB sheet, "Immune Responses to Inhalant and Food Allergens During Early Childhood", Appendix A). At the time of enrollment, and after obtaining informed consent, a questionnaire will be administered as described for the ER Study (Appendix A). In addition, five ml of blood will be drawn from both parents of children in all study groups, either at the time of enrollment, or at the first home visit, for serum IgE ab measurements by RAST to the same inhalant allergens used to test study patients. Total serum IgE levels will also be measured in the parents.

a) Measurements of IgE antibody:

At the time of enrollment and at follow-up visits, 3 to 5 ml of blood will be obtained from each patient for analysis of serum IgE ab to food and inhaled allergens and a total IgE level. In addition, serum IgE ab to Group I and Group II dust mite allergens will be measured and analyzed in relation to concentrations of these allergens in dust samples from patients' homes. Using the same allergens used for RAST, skin tests will also be carried out using the standard prick technique. Extracts used for skin tests (obtained from Hollister-Stier, Division of Miles Laboratories, Spokane, WA) will include *D. pteronyssinus*, *D. farinae*, cat hair-epithelium, cockroach

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mix, rye grass, and ragweed. In addition, intradermal skin tests (0.2 ml injected intradermally) will also be done using dust mite (100 AU/test), cat (200 PNU/test), and cockroach (200 PNU/test) extracts. Food extracts will include egg, milk, soy, peanut, fish, and wheat. Histamine phosphate (2.75 mg/ml) and sterile buffered saline in 50% glycerol will be applied as positive and negative controls, respectively, and patients will be rescheduled for testing if they have taken a short-acting antihistamine within the previous 48 hours or a long acting antihistamine within the previous 7 days. Tests will also not be applied to areas of skin treated with topical steroids in AD patients. In our experience, however, the back of AD patients is usually free of eczema. For Group IA and IB wheezing patients, skin testing will not be done when they are actively wheezing at the time of enrollment. However, shortly thereafter skin tests will be done at a follow-up appointment to the Pediatric Allergy Clinic scheduled 2 to 3 weeks after enrollment when symptoms have improved.

Information regarding the interpretation of skin tests in young children has increased over the last few years. Although dermal hyporeactivity in response to both histamine and codeine has been reported to be a problem in infants under age 6 months (72), it is clear that infants can reliably fix their own IgE on mast cells by 6-8 weeks of age and there is general agreement that after this time, skin tests resulting in a wheal size 3 mm greater in diameter than a saline control represent reliable markers of sensitization and specific IgE ab production. Results in this study will be graded on a 1+ to 4+ scale relative to the saline control. For prick tests: 3-4 mm=1+; 5-7 mm=2+; 7-10 mm=3+; and 7-10 mm+ pseudopods=4 plus. For intradermal tests: 5-8 mm=1+; 8-10 mm=2+; 10-12 mm=3+; and 10-12 mm + pseudopods=4 plus. The same inhalant extracts and criteria for interpretation will be used to prick test the parents of patients in this study. The results will be used to determine the enrollment of patients into groups IC and IIIB.

b) Analysis of mediators and eosinophils in nasal secretions and blood:
Mediators (tryptase, ECP and kinins) will be measured and eosinophils counted in nasal secretions from actively wheezing patients at the time of their enrollment in the ER (i.e. Group IA). Total blood eosinophil counts and serum ECP will be assessed in all patients at the time of enrollment and at follow-up visits. In addition, parents will be taught the simple procedure of obtaining nasal secretions from the nose of their child at home when symptoms of nasal congestion, rhinorrhea, and/or wheezing develop. The secretions are obtained using a cotton tipped swab and then transferred to a glass slide and allowed to dry. Slides are then mailed to our clinic in slide mailing packets provided to each patient and are then stained and interpreted as described above. This is a standard procedure in our allergy clinic for judging nasal eosinophilia between clinic visits, and the parents of study patients will be telephoned every 2 to 3 months by nurses following them to remind them of this assessment.

c) Assessment of exposure to indoor allergens and tobacco smoke:
Saliva for cotinine determinations will be obtained from all patients at the time of enrollment and re-assessed at each annual follow-up visit. Within 2-4 weeks after enrollment, a visit is planned to the homes of each patient. These visits will be made by Deborah Murphy and Mary Lou Hayden, nurses in our Allergy Division, who will collect vacuumed dust samples according to guidelines printed in the International Workshop report on Dust Mite Allergens and Asthma (73). These dust samples will be collected from the patient's crib (or mattress), bedroom

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floor, family room floor, and kitchen (cabinet, flooring, and counter tops). Saliva samples from family members living at home will be collected for cotinine analysis and blood will be obtained from parents not present at the time of their child's enrollment into the study. A Home Visit Questionnaire focused on the status of the home with respect to allergen sources (e.g. household pets, cockroaches, etc.) general living arrangements, and socio-economic status of the family will also be completed by the nurse (Appendix A).

Following collection, dust samples will be sieved and weighed, allergen eluted by aqueous extraction, and the supernatant stored at -20° C (73). Using allergen specific monoclonal antibody immunoassays, these samples will be analyzed for Group I and Group II mite allergens, the major cat allergen *Fel d* I, and for cockroach allergens (e.g. *Bla g* I and II). Results will be expressed in micrograms of specific allergen per gram of sieved dust, as previously described (73,74). Subsequently, homes will be visited annually in the Fall (September/October) through the duration of the study to collect additional dust samples. In the Charlottesville area, increased concentrations of mite allergen are generally found in houses during Fall months (74). Saliva samples will again be collected from the patient and family members in the home. The nurses who make these home visits will maintain records for each patient which will include copies of enrollment, home visit questionnaires, and clinic notes for follow-up visits.

d) Analysis of history and physical findings:

Patients' histories and physical findings will be recorded at the time of enrollment and at annual visits to clinic. Questionnaires to assess the home environment and the socioeconomic background of patients will be completed during the annual home visits as noted above.

Follow-up visits:

Three visits to the Pediatric Allergy Clinic at ages 2 years, 3 years, and 4 years are planned. At each visit, blood and skin test analyses will be done as described above and interval histories will be obtained focused on severity of symptoms and requirements for medications. A physical examination will also be performed. During the last year of the study, peak flow measurements will be attempted. Many children close to age 4 are able to begin using peak flow meters successfully with parental guidance.

Statistical Methods:

Statistical analysis of the relationship between IgE ab to inhalant allergens and its suspected correlates (e.g. disease state, age of patients, and allergen exposure at home) will be based largely on general linear and probability regression models (75,76). We shall also be prepared to consider non-linear relationships (77). For comparing mean differences between groups or percentages of affected patients we shall use student's t tests and chi-square analyses, respectively. We will also employ regression models to adjust our comparison of means for confounding information. In addition, we will also consider non-parametric alternatives and randomization tests (Fisher's exact test) when they appear appropriate (62). Techniques such as analysis of variance and co-variance will be used to evaluate group differences which might pertain to socio-economic status. Because immediate hypersensitivity disorders are reported to be somewhat higher in males during childhood and in black patients, we plan to identify any differences which might be attributed to race or gender in our statistical analysis. For binary, ordinal, and categorical outcomes, probability models such as logistic regression will be examined (62,75,76). Finally, for analyzing data from the

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Prospective Study, methods for the analysis of repeated measures will be employed, including techniques for the statistical modeling of trends over time (77). In addition, outcome events which occur with time will be evaluated using life table methods (78,79).

For database management and statistical analysis of data from the ER Study we have utilized PC-SAS. We do not plan to use CLINFO. Should data accumulate and exceed the capacity of PC-SAS, we are prepared to convert to the use of the mainframe SAS by uploading data sets and, thus, conduct complex statistical analyses. Dr. Abbott, who has vast experience with large data bases, is currently funded by the Honolulu Heart Program which contains large amounts of information on over 8000 individuals. He will participate in all aspects of the data analysis required for this research.

Within the Division of Biostatistics is a Biostatistics Computing Lab. Personnel within the lab provide a full range of programming skills which include database management and design and the continued monitoring and editing of collected data. An additional facility available to our project is located in the University of Virginia Academic Computing Center. This center is operating an IBM 3090-159 mainframe. Software support includes SAS, SPSS, ISML, and SQL/DS. Tape drives and disk storage are readily available. In addition, for data analysis in our laboratory, we have two IBM PC-XT and two IBM PS/2-50 computers, each with a 40 MB Hard Disk 1020 KB RAM.

Time table: We plan to enroll patients into the ER Study over the first 18 months of the grant period. For the Prospective Study, we intend to enroll half of the patients for each study group in each of the first two grant years. Thus far, patients enrolled in the ER Study have been recruited by two pediatric allergy/immunology fellows and the P.I. with supportive assistance from nurses and residents working in the ER. From July, 1993 on, we will continue to have two pediatric allergy/immunology fellows as well as our two allergy nurses who will play an important role with the recruitment and follow-up of patients in the prospective study.

Long range plans:

1) Bronchoscopies are rarely indicated in the evaluation and management of infants with bronchiolitis and, therefore, bronchial washings are not readily available to assess mediators from mast cells and eosinophils. We are in the early stages of identifying autopsy reports of children who have died from bronchiolitis or asthma. Chart reviews are planned to review diagnoses and lab data and to record medications taken. Patients on steroids at the time of death will be excluded from these analyses. After gross histological examination of lung sections for eosinophils and other infiltrating cells, more detailed studies are planned using sections from paraffin blocks to detect RSV and rhinovirus RNA via in situ hybridization with oligonucleotide probes. In addition, mAb specific for tryptase, to judge mast cell numbers, and ECP, to evaluate eosinophil infiltration, will be used to detect these mediators in lung sections from bronchiolitis and asthma patients using immunohistochemistry techniques. The facilities for carrying out in situ hybridization and immunohistochemistry experiments are well established in the Core Molecular Biology Laboratory within the Department of Pediatrics (see letter from Dr. Gomez, Appendix B).
2) With a better understanding from our studies about infants who are at risk for developing IgE ab to inhaled allergens and asthma, studies focused on mite allergen avoidance during early childhood will be planned to examine the effects of reducing allergen exposure on the timing

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of sensitization and/or attenuation of symptoms associated with inhalant allergy.

3) Since Charlottesville is a stable community, we expect to follow children enrolled in the Prospective Study for an additional 5 to 7 years. The purpose will be to examine the development or persistence of symptoms in subjects relative to their presenting diagnosis as infants and the timing of their sensitization to inhalant and food allergens.

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9. (CONTINUED) LITERATURE CITED

1. Nelson HS: The atopic diseases. Ann Allergy 1985; 55:441
2. Pollart SM, Chapman MD, Fiocco GP, Rose G, Platts-Mills TAE: Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. J Allergy Clin Immunol 1989:83:875-82.
3. O'Hollaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ, Sachs MI: Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. N Engl J Med 1991; 324:359-63.
4. Murray AB, Ferguson AC: Dust-free bedrooms in the treatment of asthmatic children with house dust or house-dust mite allergy: a controlled trial. Pediatr 1983; 71:418.
5. Platts-Mills T, Tovey E, Mitchell E, Mozarro H, Nock P, Wilkins S: Reversal of bronchial reactivity during prolonged allergen avoidance. Lancet 1982; 2:675.
6. Gergen PJ, Weiss KB: Changing patterns of asthma hospitalization among children: 1979 to 1987. JAMA; 264:1688-1692.
7. Martinez FD, Morgan WJ, Wright AL, Holber CJ, Taussig LM, GHMA: Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. N Engl J Med 1988; 319:1112-1117
8. Harlap S, Davies AM: Infant admissions to hosptial and maternal smoking. Lancet 1974; 1:529-532
9. Young S, LeSouef PN, Geilhoed GC, Stick SM, Chir B, Turner KJ, Landau LI: The influence of a family history of asthma and parental smoking on airway responsiveness in early infancy. New Eng J Med 1991; 324:1168-73.
10. Loda FA, Glezen WP, Clyde WA: Respiratory disease in group day care. Pediatrics 1972; 49:428.
11. Taussig LM: The conundrum of wheezing and airway hyper-reactivity in infancy. Pediatr. Pulmonol. 1992, 13:1-3.
12. Pullah CR, Hey EN: Wheezing, asthma, and pulmonary dysfunction ten years after infection with respiratory syncytial virus in infancy. Br Med J Clin Res 1982; 284:1665-1669.
13. Katan M, Keens TG, Lapiene JG, *et al*:Pulmonary function abnormalities in symptom free children after bronchiolitis. Pediatrics 1977; 59:683-688.
14. Samet JM, Jager IB, Speizer F: The relationship between respiratory illness in childhood and chronic air-flow obstruction in adulthood. Am Rev Respir Dis. 1983; 127: 508-523.
15. Nagayama Y, Sakurai N, Nakahara T. Allergic predisposition among infants with bronchiolitis. J Asthma. 1987; 24 (1):9-17.
16. Korppi M, Reiyonen T, *et al*: A 2 to 3 year outcome after bronchiolitis. Am J Dis Child. 1993; 147: 628-631.
17. Welliver RC, Wong DT, Sun M, Middleton E, Vaughan RS, Ogra PL: The development of respiratory syncytial virus-specific IgE and the release of histamine in nasopharyngeal secretions after infection. N Eng J Med 1981; 305:841-6.
18. Welliver RC, Wong DT, Middleton E, Sun M, McCarthy N: Role of parainfluenza virus-specific IgE in pathogenesis of croup and wheezing subsequent to infection. J Pediatr 1982; 101:889.
19. Garofalo R, Kimpen JL, Welliver RC, Ogra PL: Eosinophil degranulation in the respiratory tract during naturally acquired respiratory syncytial virus infection. J Pediatr 1992; 120:28-32.
20. Skoner DP, Fireman P, Caliguiri, Davis H: Plasma elevations of histamine and a protaglandin

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metabolite in acute bronchiolitis. Am Rev Respir Dis 1990; 142:359-364.
21. Wohl MEB, Chernick V: Bronchiolitis: State of the art. Am Rev Respir Dis 1987; 118:759-781.
22. Etzel RA: A review of the use of saliva cotinine as a marker of tobacco smoke exposure. Preventive Med 1990; 19:190.
23. Martinez FD, Cline M, Burrows B: Increased incidence of asthma in children of smoking mothers. Pediatrics 1992; 89:21-26.
24. Rowntree S, Cogswell JJ, Platts-Mills TAE, Mitchell EB: Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of allergic disease. Archives of Disease in Childhood 1985; 75:633.
25. Zeiger RS: Development and prevention of allergic disease in childhood. In: Allergy, Principles and Practice, 4th ed., Middleton E, *et al*, The CV Mosby Co., St. Louis, 1993, pg. 1141-1167.
26. Sporik R, Holgate ST, Platts-Mills TAE, Cogswell JJ: Exposure to house-dust mite allergen (*Der p* I) and the development of asthma in childhood. A prospective study. N Eng J Med 1990; 323:502-507.
27. Guillet G, Guillet MH: Natural history of sensitizations in atopic dermatitis. Arch Dermatol 1992; 128:187-192.
28. Sampson HA, McCaskil CC: Food hypersensitivity and atopic dermatitis: evaluation of 113 patients. J Pediatr 1985; 107:669.
29. Buffum WP, Settipane GA: Prognosis of asthma in childhood. Amer J Dis Child 1966; 112:214.
30. Duff AL, Wilson BB, Deuell BL, Platts-Mills TAE, Heymann PW: Early childhood inhalant allergen sensitization in atopic eczema. Submitted.
31. Langeland T: A clinical and immunological study of allergy to hen's egg white. Clin Allergy 1983; 13:371.
32. Host A, Halden S: A prospective study of cow milk allergy in Danish infants during the first 3 years of life. Allergy 1990; 45: 587-596.
33. Price GW, Duff AL, Farris AH, Burks AW, Platts-Mills TAE, Heymann PW. Sensitization (IgE antibody) to food allergens in wheezing infants and children. Submitted.
34. Croner S, Kjellman NIH: Development of atopic disease in relation to family history and cord blood IgE levels. Eleven-year follow-up in 1654 children. Pediatr Allergy Immunol 1990; 1:14.
35. Zeiger RS, Heller S, Mellon MH *et al*: Effect of combined maternal and infant food-allergen avoidance on development of atopy in early infancy: a randominzed study. J Allergy Clin Immunol 1989; 84:72.
36. Van Asperen PP, Kemp AS, Mellis CM: Skin test reactivity and clinical allergen sensitivity in infancy. J Allergy Clin Immunol 1984; 73:381.
37. Van Asperen PP, Kemp AS, Mellis CM: A prospective study of the clinical manifestations of atopic disease in infancy. Acta Paediatr Scand 1984; 73:80-85.Bullock JD, Frick OL: Mite sensitivity in house dust allergic children. Evaluation by clinical and laboratory methods. Amer J Dis Child 1972; 123:222-226.
38. Dreborg S: Skin tests used for epidemiological studies. Allergy 1989, suppl. 10:52-59.
39. Barbee R, Lebovitz M, Thompson H, Burrows B: Immediate skin test reactivity in a general population sample. Ann Intern med 1976; 84:129-133.
40. Zeiger RS: Atopy in infancy and early childhood: natural history and role of skin testing. J Allergy Clin Immunol 1989; 84:72.

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41. Kemeny DM, Richards D: Increased speed and sensitivity, and reduced sample size of a micro-radioallergosorbent test (MRAST). J Immunol Meth 1988; 108:105-113.
42. Henderson FW, Clyde WA, Collier AM, Denny FW, Senior RJ, Sheaffer CI, Conley WG, Christian RM: The etiologic and epidemiologic spectrum of bronchiolitis in pediatric practice. J Pediatr 1979; 95:183-190.
43. Berg TLO, Johansson SGO: Allergy diagnosis with the radioallergosorbent test: a comparison with the results of skin and provocation tests in an unselected group of children with asthma and hay fever. J Allergy Clin Immunol 1974; 54:209.
44. Sampson HA, James JM: Respiratory reactions induced by food challenges in children with atopic dermatitis. Pediatr Allergy Immunol. 1992; 3 (4):195-200.
45. Novembre E, de Martino M, Vierucci A. Foods and respiratory allergy. J Allergy Clin Immunol 1988; 81(5):1059-1065.
46. Onorato J, Merland N, Terral C, Michel FB, Bousquet J. Placebo-controlled double blind food challenge in asthma. J Allergy Clin Immunol 1986; 78(6):1139-1146.
47. Bousquet J, Chaney P, Lacoste JY, Barneon G, Ghavanian N, Enander I, Venge P, Ahlstedt S, Simony-Lafontaine J, Goddard P, Michel FB: Eosinophilic inflammation in asthma. New Eng J Med 1990; 323:1033-9.
48. Lim D: Eosinophil leukocytes. In: Nasal Allergy, ed. Niels Mygind, Blackwell Scientific Publications, 2nd ed. 1979, pg. 170-181.
49. Proud D, Naclerio RM, Gwaltney JM, Hendley JO: Kinins are generated in nasal secretions during natural rhinovirus colds. J Infect Dis 1987; 157:133.
50. Proud D, Reynolds CJ, La Capra S, *et al*: Nasal provocation with brady-kinin induced symptoms of rhinitis and a sore throat. Am Rev Resp Dis 1988; 137:613-616.
51. Langone JJ, Van Vunakis: Radioimmunoassay of nicotine, cotinine, and -(3-pyridyl)-oxo-N-methylbutyramide. Methods in Enzymology 1982; 84:628.
52. Deuell BL, Wilson BB, Heymann PW, Platts-Mills TAE: Atopic dermatitis (A.D.): the role of dust mite allergy and monitoring of disease activity by assay of IgG in skin scales. J Allergy Clin Immunol 1991; 87:237
53. Adinoff Ad, Tellez P, Clark RAF: Atopic dermatitis and aeroallergen contact sensitivity. J Allergy Clin Immunol 1988; 81:736-742.
54. Hanifin JM, Rajke G: Diagnostic features of atopic dermatitis. Acta Derm Venerol [Supp.] 1980; 92:44-47.
55. Chapman MD, Platts-Mills TAE: Purification and characterization of the major allergen of *Dermatophagoides pteronyssinus* - antigen P1. J Immunol 1980; 125:527-592.
56. Heymann PW, Chapman MD, Platts-Mills TAE: Antigen *Der f* I from the dust mite *Derma tophagoides farinae*: Structural comparison with antigen *Der p* from *D. pteronyssinus* and epitope specificity for murine IgG and human IgE antibodies. J Immunol 1986; 137:2841-2847.
57. Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TAE: Antigenic and structural analysis of Group II allergens (*Der f* II and *Der p* II) from house dust mites (Dermatophagoides spp.). J Allergy Clin Immunol 1989; 83:1055-1067.
58. Calkhoven PG, Aalbers M, Schilte PPM, Yutema BL, Griffioen, RW, van Nierop JC, Oranje AP, Aalberse RC: Newly generated IgE antibodies to *Dermatophagoides pteronyssinus* in children are directed against components distinct from *Der p* I and *Der p* II. J Allergy Clin Immunol 1991; 88:265-271.
59. Heymann PW, Chapman MD: Recent progress in mite allergen immunochemistry. Clin Rev

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Allergy 1990; 8:51-68.
60. Chapman MD, Heymann PW, Wilkins SR and Platts-Mills TAE: Monoclonal immunoassays for major dust mite (*Dermatophagoides*) allergens, *Der p* I and *Der f* I, and quantitative analysis of the allergen content of mite and house dust extracts. J Allergy Clin Immunol 1987; 80:184-194.
61. Heymann P, Chapman M, Whelchel A, Platts-Mills T: Concentrations of two major allergens in *Dermatophagoides* mite extracts. J Allergy Clin Immunol 1988; 81:268.
62. Rosner B: Fundamentals of Biostatistics (3rd ed.). Boston, PWS-Kent Publishing Co., 1990.
63. Spector SL: Definition of asthma. ATS News, Fall 1982, Vol. 8, pg 5.
64. Jarvis MJ, McNeill AD, Bryant A, Russell MAH: Factors determining exposure to passive smoking in young adults living at home: quantitative analysis using saliva cotinine concentrations. Int J Epidemiol 1991; 20-126-131.
65. Proud D, Bailey GS, Naclerio RM, Reynolds CJ, Cruz AA, Eggleston PA, Lichtenstein LM, Togias AG: Tryptase and histamine as markers to evaluate mast cell activation during repsonses to nasal challenge with allergen, cold, dry air, and hyperosmolar solutions. J Allergy Clin Immunol 1992; 89:1098-1110.
66. Proud D, Togias A, Naclerio RM, Crush SA, Norman PS, Lichtenstein LM: Kinins are generated in vivo following nasal airway challenge of allergic individuals with allergen. J Clin. Invest. 1983; 72:1678-1685.
67. Naclerio RM, Proud D, Lichtenstein LM, Kagey-Sobotka A, Hendley JO, Sorrentino J, Gwaltney JM: Kinins are generated during experimental rhinovirus colds. J Infectious Dis 1988; 157:133-142.
68. Wenz B, Ramirez MA, Burns ER: The H*1 Hematology Analyzer. Arch Pathol Lab Med
69. Welliver RC, Sun M, Rinaldo D, Ogra PL: Respiratory syncytial virus-specific IgE responses following infection: evidence for a predominantly mucosal response. Pediatr. Research 1985; 19:420-424. 1987; 111:521-524.
70. Ueba O: Respiratory syncytial virus. I. Concentration and Purification of the infectious virus. Acta Med Okayama 1978; 32:265-72.
71. Platts-Mills TAE, Maur RK von, Ishizaka K, Norma PS, Lichtenstein LM 1976. IgA and IgG anti-ragweed antibodies in nasal secretions. Quantitative measurements of antbodies and correlation with the inhibition of histamine release. J Clin Invest. 57:1041-1050.
72. Menardo JL, Bonsquet J, Rodiere M, Astric J, Michel FB: Skin test reactivity in infancy. J Allergy Clin Immunol 1985; 75:646.
73. Report of the second International Workshop: Dust mite allergens and asthma. Pub: The UCB Institute of Allergy, Brussels, Belgium.
74. Platts-Mills T, Hayden M, Chapman M, Wilkins S: Seasonal variations in dust mite and grass pollen allergens. J Allergy Clin Immunol 1987; 79:781.
75. Netter J, Wasserman W: Applied Linear Statistical Models. Homewood, IL, Richard D. Irwin, 1974.
76. McCullough P, Nelder JA: Generalized Linear Models. New York, Chapman and Hall, 1983.
77. Gallant AR: Nonlinear Statistical Models. New York, John Wiley and Sons, 1987.
78. Cox DR: Analysis of Binary Data. New York, Chapman and Hall, 1970.
79. Agresti A: Analysis of Ordinal Categorical Data. New York, John Wiley and Sons, 1984.

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10. AVAILABLE FACILITIES AND RESOURCES

FACILITIES:

Laboratory: Laboratory work for this project will be carried out in the Division of Allergy and Clinical Immunology. Research laboratories are located in Building MR-4, UVA Health Sciences Center. The division has 2,800 sq. ft. of space including individual laboratories for Drs. Heymann, Platts-Mills, and Chapman, a separate radioisotope lab of 300 sq. ft. and an adjacent cold room (4° C) of 70 sq. ft.

Clinical: The Pediatric Allergy Clinic is located in the Primary Care Center at UVA. During the last 12 months, 584 patients were seen, 62% for asthma and 14% for atopic dermatitis. Four examination rooms are available for patient evaluation and two nurses assist with skin testing, pulmonary function testing and nasal smear preparation. (See other clinical resources below.)

Animal: Mice required for raising ascites will be housed in the vivarium on the basement level of the MR-4 Bldg. and in the Jordan Hall Medical School building adjacent to the Lymphocyte Culture Center. The vivarium is part of the Division of Comparative Medicine and is fully accredited by the American Association for Accreditation of Laboratory Animal Care.

Computer: The computer resources, facilities, and environment for this project are described in Section 4 (Research Design and Methods). In addition, the UVA Academic Computing Center, which operates an IBM 3090-159 mainframe, is available to our project. Software support includes SAS, SPSS, ISML, and SQL/DS. Tape drives and disk storage are available. Two IBM PC-XT and two IBM PS/2-50 computers are located in the Allergy Research Lab, each with a 40 MB Hard Disk 1020 KB RAM.

Office: Office space for the P.I and for Ms. Farris is located within the Allergy Laboratory in Bldg. MR-4. Secretarial space is located on the second floor of Bldg. MR-4. Office space for nurses involved in this project is located adjacent to the clinic.

Other: The Lymphocyte Culture (Hybridoma) Center is located in the Jordan Hall Medical School at UVA immediately opposite the MR-4 Building.

MAJOR EQUIPMENT:

The following items of equipment are all located within the Division of Allergy Laboratories and are pertinent to this project: Micromedic 4-channel gamma counter, Fisher Scientific 20-liter water bath, Beckman J-6B Centrifuge, Beckman J2-21 High Speed Centrifuge, -70° C freezer, Labconco Freeze Dryer, Flow Multiscan ELISA reader and plate washer, fume hood, LKB constant wattage power supply, Pharmacia Phast System, multi-tube vortexer, and chromatography refrigerator.

ADDITIONAL INFORMATION:

The Pediatric Emergency Room at UVA is located in the ground floor of the main hospital across from the MR-4 Bldg. During the last year (1991) 15,034 patients were seen of which 489 (3.2%) were treated for asthma or bronchiolitis.

The Dermatology and Immunodermatology clinics are located in the Primary Care Center Bldg. one floor directly above the Pediatric Allergy Clinic. Last year 1214 patients with atopic dermatitis

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were seen in these clinics approximately 10% of whom were infants. Dr. Barbara Wilson, who will facilitate the recruitment of patients from these clinics, has an office adjacent to the clinic area (see support letter).

The General Pediatric Outpatient clinic is located in the Primary Care Center Bldg. on the same floor, and adjacent to, the Pediatric Allergy Clinic. Last year (1991) 12, 281 patients were seen in this clinic. Forty-two percent of the visits were for well child care and 147 children were treated for eczema.

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11. OTHER SUPPORT FOR PRINCIPLE INVESTIGATOR:

PETER W. HEYMANN

Source and identifying no: NIH-RFA No. AI-92-07- Active. **P.I.:** Dr. Thomas A.E. Platts-Mills **Title:** UVA Asthma and Allergic Diseases Center **Role on project:** Co-Investigator **% Effort:** 15%
Dates and costs of entire project: 7/1/93 - 6/30/98; Direct costs $500,000 (Project 3) **Dates and costs of current year:** 7/1/93 - 6/30/94; Direct costs $100,000 (Project 3) **Specific aims of project:** Project 3. Main objective will be to investigate CD4+ lymphocyte responses to respiratory syncytial virus (RSV) antigens in humans with asthma and controls. **Describe scientific and budgetary overlap:** None. **Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)** None.

Source and identifying no: NIAID R37 AI-20565-09 - Active. **P.I.:** Dr. Thomas A.E. Platts-Mills **Title:** Dust mite, cockroach and cat allergens in houses: Asthma **Role on project:** Co-investigator. **% Effort: 10% Dates and costs of entire project:** 7/1/92 - 4/30/97; direct costs $1,056,243 **Dates and costs of current year:** 5/1/93 - 4/30/94; direct costs $208,939 **Specific aims of project:** Epidemiological and immunological studies of allergens produced by the dust mite, cockroach and cat in relation to the etiology of asthma. Assessment of allergen avoidance regimens for dust mite and cat allergen, e.g. use of acaricides, air filtration and vacuum cleaners.
Describe scientific and budgetary overlap: Examining the relationship between allergen exposure and sensitization in early childhood is unique to the current proposal. There is overlap in the area of analyzing exposure to mite, cat and cockroach allergens in dust samples. **Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)** None.

Source and identifying no.: NIH-RO1 AI-30840 - Active. **P.I.:** Dr. Thomas A.E. Platts-Mills Title: Trichophyton and Aspergillus: Immunology and Lung Disease **Role on the project:** Co-investigator. **% Effort:** 5% **Dates and costs of entire project:** 3/1/91-2/28/94 $417,255. **Dates and costs of current project:**3/1/93-2/28/94 $143,707. **Specific aims of project:** Funds were awarded to study the immunochemistry of allergens produced by these fungi and their role in allergic disease. My effort accounts for studies of immune responses to Aspergillus allergens in patients with cystic fibrosis. **Describe scientific and budgetary overlap:** None.
Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.) None.

Source and identifying no.: NIH, RR00847 - Active. **P.I.:** Robert M. Carey
Title: University of Virginia General Clinical Research Center **Role on project:** N/A **% Effort:**0% **Dates and costs of entire project:** Grant Period: December, 1985 to November, 1995 **Dates and costs of current year:**
Specific aims of project: Funds from this grant ($4,500 through June 30, 1994) are available to cover the costs of RSV antigen detection in nasal aspirates and CBC analyses for patients enrolled in the ER Study described in this grant. Costs for these tests have not been included in the budget for this grant proposal. Data management and support, including programming, are also available to our project through this grant.

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Describe scientific and budgetary overlap: None.
Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.) None.

OTHER SUPPORT FOR KEY PERSONNEL:

Thomas A.E. Platts-Mills

Source and identifying no.: NIH, RFA No. AI-92-07 - Active. **P.I.:** Thomas Platts-Mills
Title: University of Virginia Asthma and Allergic Diseases Center **Role on project:** Principal Investigator **% Effort:** 20% **Dates and costs of entire project:**
Project 4: 7/1/93-6/30/98; direct costs $450,000 Project 5: 7/1/93-6/30/98; direct costs $375,000
Dates and costs of current year: Project 4: 7/1/93-6/30/94; direct costs $90,000
Project 5: 7/1/93-6/30/94; direct costs $75,000
Specific aims of project: Project 4: School study focused on inner city school with 50% African American children and high rates of poverty. The project is essentially to extend school studies into the inner city. Project 5: Avoidance studies on children presenting with acute asthma. This is an outreach study which is planned to be carried out in collaboration with the other centers. **Describe scientific and budgetary overlap:** None.
Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.) None.

Source and identifying no: NIAID R37 AI-20565 - Active. **P.I.:** Dr. Thomas A.E. Platts-Mills **Title:** Dust mite, cockroach and cat allergens in houses: Asthma **Role on project:** Principal investigator. **% Effort: 20% Dates and costs of entire project:** 7/1/92 - 4/30/97; direct costs $1,056,243 **Dates and costs of current year:** 5/1/93 - 4/30/94; direct costs $208,939 **Specific aims of project:** Epidemiological studies on the role of indoor allergens in asthma. Detailed studies of the factors influencing airborne levels of dust mite and cat allergens including acaricides, air filtration and vacuum cleaners. **Describe scientific and budgetary overlap:** Examining the relationship between allergen exposure and sensitization in early childhood is unique to the current proposal. There is overlap in the area of analyzing exposure to mite, cat and cockroach allergens in dust samples. **Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)** None.

Source and identifying no.: NIAID, R01-AI-32557-01 - Active. **P.I.:** Martin D. Chapman
Title: Cockroach allergens: Immunochemistry and Allergic Disease **Role on project:** Investigator
% Effort: 5% **Dates and costs of entire project:** 7/1/93 - 6/30/96; Direct costs $407,502
Dates and costs of current year: 7/1/93 - 6/30/94; Direct costs $119,689 **Specific aims of project:** Antigen analysis of *B. germanica* and *P. americana* allergens; molecular cloning of *B. germanica* allergens. **Describe scientific and budgetary overlap:** None. **Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)** None.

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Robert D. Abbott

Source and identifying no.: NIH RR00847-19 - Active. **P.I.:** Robert M. Carey
Title: University of Virginia General Clinical Research Center **Role on project:** biostatistician
% Effort: 30% **Dates and costs of entire project:** 12/1/90 - 11/30/95; Direct costs $11,001,929
Dates and costs of current year: 12/1/91 - 11/30/92; Direct costs $2,177,132 **Specific aims of project:** A Program to support approved general clinical research **Describe scientific and budgetary overlap:** There is no overlap. This is a request to increase current levels of support for biostatistics. **Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)** None.

Source and identifying no.: Kuakini Medical Center SC-07-8-91UV - Active **P.I.:** Robert D. Abbott
Title: Biostatistics Core for the Honolulu Heart Program **Role on project:** Director of the Biostatistics Core **% Effort:** 25% **Dates and costs of entire project:** 8/16/91 - 12/31/94; Direct costs $399,898
Dates and costs of current year: 8/16/91 - 8/15/92; Direct costs $132,986 **Specific aims of project:** To provide biostatistical support for the Honolulu Heart Program **Describe scientific and budgetary overlap:** There is no overlap **Describe adjustments you will make if the present application is funded (budget, % effort, aims, etc.)** None.

Source and identifying no.: NHLBI 1R01-HL-43611 - Active **P.I.:** John B. Schorling
Title: A trial of smoking cessation programs in black churches **Role on project:** biostatistician
% Effort: 10% **Dates and costs of entire project:** 7/1/91 - 6/30/94; Direct costs $488,000
Dates and costs of current year: 7/1/91 - 6/30/92; Direct costs $216,868 **Specific aims of project:** A project to investigate smoking cessation programs in black churches **Describe scientific and budgetary overlap:** There is no overlap **Describe adjustments you will make if the present application 1is funded (budget, % effort1, aims, etc.)** None.

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Principal Investigator: HEYMANN, PETER W.

12. BUDGET.

Detailed specific needs for the first 12-month period. Estimate category sub-totals for 2nd and 3rd periods, if required. Append justifications.

(a) Salaries. List personnel by name and title. Indicate individuals % time to be spent on this project.	$ 1st period	$ 2nd period	$ 3rd period
% Professional:			
35% Peter W. Heymann Associate Professor, Pediatrics			
5% Thomas A.E. Platts-Mills Professor, Medicine and Microbiology			
7% Robert D. Abbott Professor, Division Head, Biostatistics			
% Technical:			
100% A. Hollis Parris Research Assistant			
80% Deborah D. Murphy Nurse Clinician			
20% Mary Lou Hayden Nurse Clinician			
% Other:			
Fringe benefits payable at institution's rate* * See Justifications.			
Category (a) Sub-Total			
(b) Consultants (per diem, travel & expenses):			
Category (b) Sub-Total			
(c) Supplies & Expense:			
Consumables (by category) Disposable plastics Chemicals, glassware, gels and small equipment Radioisotopes Dialysis tubing			
Animals and related costs			
Other expenses (itemize) Viral cultures Patient care costs (see justifications below)			
Category (c) Sub-Total			
(d) Travel Expenses: Category (d) Sub-Total			
(e) Alterations and Renovations: Category (e) Sub-Total			
(f) Sub-contracts Category (f) Sub-Total			
(g) Equipment Gateway 2000/4DX-33V computer			
Category (g) Sub-Total			
(h) TOTAL DIRECT COSTS			
(i) Indirect costs not to exceed 25% of the sum of (a) thru (f):			
(j) TOTAL PROJECT COSTS			

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12. BUDGET (JUSTIFICATIONS)

(a) **Salaries:**

Funds are requested to support 35% of the Principle Investigator's salary which will account for effort in experimental work and design, supervising laboratory personnel, data analysis, publishing manuscripts, and presenting results at national meetings. Support for Dr. Thomas Platts-Mills reflects his ongoing involvement with supervising the collection and data analysis of dust samples from homes of patients enrolled in the Prospective Study. Dr. Robert Abbott is Head of the Division of Biostatistics at the University of Virginia. Support for Dr. Abbott reflects his participation in all aspects of statistical modeling and data analysis required for this research. Technical support will be provided by Holly Farris who has been working with the P.I. for the past 4 years. Ms. Farris is very experienced with immunochemical techniques, including immunoassays, allergen purification, and the RIA for cotinine determinations. Deborah Murphy, R.N., has worked with the P.I. for the last six years as the Head Nurse in the Pediatric Allergy Clinic. She will devote 80% of her time to this project and will play a central role in facilitating the recruitment of patients and maintaining records for each patient followed prospectively. Ms. Murphy will also be responsible for skin testing patients and has extensive experience working with infants and young children. Along with Ms. Murphy, Ms. Hayden will assist in visiting patient's homes to collect dust samples. Note: Fringe benefit rates for faculty and research assistants at the University of Virginia are: 23.5%, 24.5%, and 25% for the first, second, and third grant periods, respectively. For the nurse clinicians the rates as classified employees are 30%, 32.5%, and 33.5% respectively.

(b) **Consultants:**

Dr. David Proud, whose laboratory at the Johns Hopkins Asthma and Allergy Center will be monitoring nasal secretions for tryptase and kinins, will act as a consultant regarding mediator analysis. Dr. Frederick Hayden will be a consultant for data obtained from viral cultures and detection of rhinovirus RNA by PCR analysis. No consultant fees are required.

(c) **Supplies & Expenses:**

Consumable and animal costs.

Costs for supplies are requested for radioisotopes, glassware, disposable plastics, chemicals, dialysis tubing, gels for affinity purifying dust mite allergens and for gel filtration, and small items of equipment (e.g. pipettors, stir plates, and vortex mixers). Animal costs will cover the production of ascites containing mAb for use in two-site immunoassays for measuring mite allergens in dust samples and for affinity purifying mite allergens to be used in antigen binding and immunoabsorption experiments.

Other expenses:

Viral Cultures. Viral cultures of nasal aspirates from wheezing and control patients in the ER Study will be done in Dr. Fred Hayden's laboratory at the University of Virginia and will cost $65 per patient. Funds to cover costs for rhinovirus RNA detection by PCR analysis in Dr. Hayden's laboratory are already available from our division. We anticipate enrolling 135 patients who will

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require viral cultures during the first grant year and an additional 95 patients in the second grant year. Costs for RSV antigen detection and CBC with differential counts will be covered through institutional research funds from the University of Virginia General Clinical Research Center (NIH Grant # RR00847).

Patient care costs: Patient participation costs include $20 to be paid to each patient (or parent) at the time of enrollment into the ER or Prospective Studies. We have also added $1000 to cover telephone charges to track children enrolled in the Prospective Study. We anticipate enrolling 180 patients into these studies during the first grant year and an additional 140 patients in the second grant year.

(g) **Equipment:**

The Gateway 2000/4DX-33V computer will be needed and sufficient for data base management in our laboratory as we continue to enroll patients in our ER and Prospective Studies and generate more data.

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13. BIOGRAPHICAL SKETCH

Name: Peter W. Heymann, M.D.
Title: Associate Professor of Pediatrics
Institution: University of Virginia
Date of birth: REDACTED

Education:

Institution and Location	Degree	Year	Field of Study
Washington University (St. Louis)	B.S.	1966	Biology
John Carroll University (Cleveland)	M.S.	1969	Biochemistry
Case Western Reserve University (Cleveland)	M.D.	1973	Medical Degree
Rainbow Babies & Children's Hosp. (Cleveland)	P.L. 1,2,3	1976	Pediatrics
Harvard University (Boston)	M.PH.	1978	Child Health

Professional experience and honors:
-Alpha Omega Alpha, Cleveland, OH 1976.
-Medical Director, Children & Youth Program (C & Y), Charlottesville, Va 1978-82.
-Director, Student Teaching Program in Pediatrics, University of Virginia, 1981-83.
-Fellow in Allergy and Immunology, University of Virginia, 1983-87.
-Assistant Professor of Pediatrics, University of Virginia, 1987-1990.
-Associate Professor of Pediatrics, University of Virginia, 1990.

Major research interests:
(1) Development of immune responses to food and inhaled allergens during early childhood.
(2) Major risk factors for wheezing in young children with bronchiolitis and asthma.

Selected Publications:

Chapman MD, Heymann PW, Sutherland WM, Platts-Mills TAE: Recognition of species specific and cross-reacting antigenic determinants on house dust mite (*Dermatophagoides*) allergens using monoclonal antibodies. Int Archs Allergy Appl Immun. 77:166-168, 1985.

Platts-Mills TAE, Heymann PW, Chapman MD, Hayden ML, Wilkins SR: Cross-reacting and species specific determinants on a major allergen from *Dermatophagoides pteronyssinus* and *D. Farinae*: Development of a radioimmunoassay for "Antigen P_1 Equivalent" in house dust and dust mite extracts. J Allergy Clin Immunol. 78:398, 1986.

Heymann PW, Chapman MD, Platts-Mills TAE: Antigen *Der f* I from the dust mite *Dermatophagoides farinae*: Structural comparison with antigen *Der p* I from *D. pteronyssinus* and epitope specificity for murine IgG and human IgE antibodies. J Immunol, 137:2841-2847, 1986.

Chapman MD, Heymann PW, Wilkins SR, Brown MJ, Platts-Mills, TAE: Monoclonal Immunoassays of major dust mite (*Dermatophagoides*) allergens, *Der p* I and *Der f* I from mites of the genus *Dermatophagoides*. J Immunol, 139:1479-1484, 1987.

Platts-Mills TAE, Mitchell EB, Chapman MD, Heymann, PW: Dust mite allergy: Its clinical significance. Hospital Practice, 91-100, September, 1987.

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Platts-Mills TAE, Heymann, PW: Natural History of Asthma. In: Highlights of Asthmology. Bosquet J, Godard P, Michel FB. Pub. Springer-Verlag, pp. 7-18, 1987.

Heymann, PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TAE: Antigenic and structural analysis of Group II allergens (*Der f* II and *Der p* II) from house dust mites (*Dermatophagoides spp.*) J Allergy Clin Immunol, 83:1055-1067, 1989.

Chapman MD, Heymann, PW, Pollart SM, Platts-Mills TAE: Allergen standardization using monoclonal antibodies to major allergens. In: Proceedings of the XIII International Congress of Allergology and Clinical Immunology (Montreux, Switzerland); Eds: Hans Huber Publ; pp. 407-409, 1989.

Platts-Mills TAE, Pollart SM, Luczynska CM, Chapman MD, Heymann, PW: The role of indoor allergens in asthma. In: Progress in Allergy and Clinical Immunology. Ed: WJ Pichler et al; Pub: Hogrefe and Huber Publishers, Toronto; pp. 279-285, 1989.

Heymann, PW, Chapman MD: Recent progress in mite allergen immunochemistry. Clin Rev Allergy, 8:51-68, 1990.

Chapman MD, Pollart SM, Heymann, PW: Allergens. In: Childhood Allergic Rhinitis and Sinusitis. Ed. DG Tinkleman. Pub. Marcel Dekker, Inc., New York, NY, pp. 37-62, 1990.

Heymann, PW, Murphy DD: Allergy Evaluation and Treatment Procedures. Ed. JA Lohr, Pub. J.B, Lippincott Co., Chapt. 19, 240-257, 1991.

Platts-Mills TAE, Ward G, Sporik R, Gelber LE, Chapman MD, Heymann PW: Epidemiology of the relationship between exposure to indoor allergens and asthma. Int Arch Appl Immunol, 443, 1991.

Platts-Mills TAE, Chapman MD, Mitchell B, Heymann PW, Deuell BL: The role of inhalant allergens in atopic dermatitis. In: Handbook of Atopic Eczema. Ed: J.Ring;Pub:Springer Verlag, Berlin. In press.

Duff AL, Pomeranz E, Gelber LE, Price GW, Farris AH, Christoph RA, Hayden FG, Platts-Mills TAE,Heymann PW: Risk factors for acute wheezing in children: viruses, passive smoke, and IgE antibodies to inhalant allergens. Pediatr. In press.

Ingram JM, Heymann PW: Environmental controls in the management of asthma. Immunology and Asthma Clinics of N.A. (In press).

El-Dahr JM, Fink R, Selden R, Arruda LK, Platts-Mills TAE, Heymann PW. Development of immune responses to Aspergillis at an early age in children with cystic fibrosis. Submitted.

Ovsyannikova IG, Vailes LD, Ying L, Heymann PW, Chapman MD. Monoclonal antibodies to Group II *Dermatophagoides* spp. allergens: Murine immune response, epitope analysis and development of a two site ELISA. Submitted.

Duff AL, Wilson BB, Deuell BL, Platts-Mills TAE, Heymann PW. Early childhood inhalant allergen sensitization in atopic eczema. Submitted.

Price GW, Duff AL, Farris AH, Burks AW, Platts-Mills TAE, Heymann PW. Sensitization (IgE antibody) to food allergens in wheezing infants and children. Submitted.

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BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE
Thomas A.E. Platts-Mills	Professor of Medicine; Head, Allergy & Clin Immunol

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Bailliol College Oxford University	BA	1963	Animal Physiology
St. Thomas Hospital, London	BM BCh(Oxon)	1967	Medical Degree
Royal College of Physicians, London	MRCP	1971	Internal Medicine
Johns Hopkins University	Fellowship	1971-74	Immunology
London University	PhD	1983	Medicine

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

- Medical Registrar, New Market General Hospital, 1969-1971.
- Medical Research Council (UK) and Northwich Park Hospital, 1974-1982. Permanent Staff Member and Honorary Consultant Physician, 1979-1982.
- Fellow, Royal College of Physicians, London, 1982.
- Professor of Medicine and Head, Division of Allergy and Clinical Immunology, University of Virginia School of Medicine, 1982 - Present.
- Editorial Board: Clin. Exp. Immunol., Methods in Immunol., Clinical Allergy, J. Allergy Clin. Imm.
- Member, Immunological Sciences Study Section, National Institutes of Health, 1988.
- Member, Association of American Physicians, 1992.

Representative Publications from over 90 Published in Reviewed Journals.

Chapman, MD and Platts-Mills, TAE (1980). Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P1. J.Immunol. 125:587-592.

Tovey, ER, Chapman, MD, Wells, CW and Platts-Mills, TAE (1981). The distribution of dust mite allergen in the houses of patients with asthma. Am. Rev. Resp. Dis. 124:630-635.

Tovey, ER, Chapman, MD and Platts-Mills, TAE (1981). Mite faeces are a major source of house dust allergens. Nature 289:592-593.

Platts-Mills, TAE; Tovey, ER; Mitchell, EB; Moszoro, H; Nock, P; and Wilkins, SR (1982). Reduction of bronchial hyper-reactivity during prolonged allergen avoidance. The Lancet, ii:675-678.

Mitchell, EB, Crow, J, Chapman, MD, Jouhal, SS, Pope, FM and Platts-Mills, TAE (1982). Allergen induced eczematous patch test sites, containing basophils, in atopic dermatitis. Lancet i:127-130.

Chapman, MD; Sutherland, WM; and Platts-Mills, TAE (1984). Recognition of two *Dermatophagoides pteronyssinus* specific epitopes on Antigen P1 using monoclonal antibodies. J. Immunol.133:2488-95.

Rawle, FC; Mitchell, EB; and Platts-Mills, TAE (1984). T cell responses to the major allergen from the house dust mite *Dermatophagoides pteronyssinus*, Antigen P_1. J. Immunol. 133:195-201.

Mitchell, EB, Crow, J, Rowntree, S, Webster, ADB and Platts-Mills, TAE (1984). Cutaneous basophil hypersensitivity to inhalant allergens. J. Invest. Dermatol. 83:290-295.

Ford, A; Seagroatt, V; Platts-Mills, TAE; and Lowenstein, H (1985). A collaborative study on the first international standard of *D.pteronyssinus*. J. Allergy Clin. Immunol. 75:676-686.

Mitchell, EB, Crow, J, Williams, G and Platts-Mills, TAE (1986). Increase in skin mast cells following chronic house dust mite exposure. Brit. J. Dermatol. 114:65-73.

Platts-Mills, TAE, Heymann, PW, Longbottom, J and Wilkins, SR (1986). Airborne allergens associated with asthma. J. Allergy 77:850-857.

Heymann, PW, Chapman, MD and Platts-Mills, TAE (1986). Antigen *Der f* I from the dust mite *Dermatophagoides farinae*. J. Immunol. 137:2841-2847.

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Platts-Mills, TAE; Hayden, ML; Chapman, MD; and Wilkins, SR (1987). Seasonal variation in dust mite and grass pollen allergens. J. Allergy Clin. Immunol 79:781-791.

Platts-Mills, TAE; Longbottom, J; Edwards, J; *et al.* (1987). Occupational asthma and rhinitis related to laboratory rats. J. Allergy Clin. Immunol. 79:505-515.

Platts-Mills, TAE and Chapman, MD (1988). Dust mites: Immunology, allergic disease, and environmental control. J. Allergy Clin. Immunol. 80:755-775.

Chapman, MD; Aalberse, RC; Brown, MJ; Platts-Mills, TAE (1988). Monoclonal antibodies to the major feline allergen *Fel d* I. J. Immunol. 140:812-818.

Pollart, SM; Reid, MJ; Brown, M; Kwasnicki, M; Fling, JA; Chapman, MD; Platts-Mills, TAE (1988). Epidemiology of Emergency Room asthma in Northern California. J. Allergy Clin. Immunol. 82:224.

Luczynska, CM; Arruda, LK; Platts-Mills, TAE; Miller, JD; Lopez, M; Chapman, MD (1989). A two-site monoclonal antibody ELISA for *Der p* I and *Der f* I. J. Immunol. Meth. 118:227-235.

Heymann, PW; Chapman, MD; Aalberse, RC; Fox, JW; Platts-Mills, TAE (1989). Antigenic and structural analysis of Group II allergens. J. Allergy Clin. Immunol. 83:1055-1067.

Platts-Mills, TAE and de Weck, A (1989). Dust mite allergens and asthma -- A world wide problem. Report of an International Workshop, Bad Kreuznach, FRG, Sept.'87. J All Clin Imm 83:416-427.

Ward, GW; Karlsson, G; Rose, G; Platts-Mills, TAE (1989). Trichophyton asthma: specific sensitization of the bronchi and upper airways to a dermatophyte antigen. The Lancet i:859-862.

Pollart, SM; Chapman, MD; Fiocco, GP; Rose, G; Platts-Mills, TAE (1989). Epidemiology of acute asthma. J. Allergy Clin. Immunol. 83:875-882.

Luczynska, CM; Li, Y; Chapman, MD; Platts-Mills, TAE (1990). Airborne concentrations of allergen derived from domestic cats (*Felis domesticus*). Am. Rev. Resp. Dis. 141:361-367.

Sporik, R; Holgate, ST; Platts-Mills, TAE; Cogswell JJ (1990). House dust mite allergen (*Der p* I) exposure and the development of sensitization and asthma in childhood: A prospective study. N. Eng. J. Med. 323:502-507.

Arruda, LK; Platts-Mills, TAE; Fox, JW; Chapman, MD (1990). *Aspergillus fumigatus* allergen I, a major IgE binding protein, is a member of the mitogillin family of cytotoxins. J Exp Med 172:1529-32.

Pollart, SM; Smith, TF; Morris, EC; Gelber, LE; Platts-Mills, TAE; Chapman, MD (1991). Environmental exposure to cockroach allergens. J. Allergy Clin. Immunol. 87:505-510.

Pollart, SM; Mullins, DE; Vailes, LD; Hayden, ML; Platts-Mills, TAE; Sutherland, WM; Chapman, MD (1991). Identification of cockroach allergens using mAb. J. Allergy Clin. Immunol. 87:511-521.

De Blay, F; Chapman, MD; Platts-Mills, TAE (1991). Airborne cat allergen (*Fel d* I): Environmental control with the cat *in situ*. Amer. Rev. Resp. Dis. 143:1334-1339.

Deuell BL, Arruda LK, Hayden ML, Chapman MD, Platts-Mills TAE (1991). *Trichophyton tonsurans* allergen I (*Tri t* I). J. Immunol., 147:96-101.

De Blay F, Heymann PW, Chapman MD, Platts-Mills TAE (1991). Comparison of Group II allergens with Group I mite allergen and cat allergen *Fel d* I. J. Allergy Clin. Immunol.; 88:919-926.

Hayden ML, Rose G, Diduch KB, Domson P, Chapman MD, Heymann PW, Platts-Mills TAE (1992). Reduction of dust mite allergens in carpets using benzyl benzoate in the form of a wet powder. J. Allergy Clin. Immunol.; 89:536-545.

Arlian LG, Bernstein D, Platts-Mills TAE, *et al.* (1992). Prevalence of dust mites in the homes of people with asthma living in eight different geographic areas of the United States. J Allergy Clin Immunol; 90:292-300.

Arruda LK, Platts-Mills TAE, Longbottom JL, El-Dahr JM, Chapman MD (1992). *Aspergillus fumigatus*: Identification of 16kd, 18kd and 45kd antigens recognized by human IgG and IgE antibodies and murine monoclonal antibodies. J Allergy Clin Immunol; 87:1166-1176.

Call RS, Smith TF, Morris E, Chapman MD, Platts-Mills TAE (1992). Risk factors for asthma in inner city children. J. Pediatr.; 121:862-866

Gelber LE, Seltzer LH, Bouzoukis JK, Pollart SM, Chapman MD, Platts-Mills TAE (1993). Sensitization and exposure to indoor allergens as risk factors for asthma among patients presenting to hospital. Amer Rev Resp Dis; 147:573-578.

Woodfolk JA, Luczynska CM, de Blay F, Chapman MD, Platts-Mills TAE (1993). The effect of vacuum cleaners on the concentration and particle size distribution of airborne cat allergen. J Allergy Clin Immunol; 91:829-837.

Sporik RB, Arruda LK, Woodfolk J, Chapman MD, Platts-Mills TAE (1993). Environmental exposure to *Aspergillus fumigatus* allergen (*Asp f* I). Clin Exp Allergy 23:326-331.

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BIOGRAPHICAL SKETCH

Give the following information for the key personnel and consultants and collaborators. Begin with the principal investigator/program director. Photocopy this page for each person.

NAME	POSITION TITLE	
Robert D. Abbott	Professor and Head	April 21, 1950

EDUCATION *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE	YEAR CONFERRED	FIELD OF STUDY
Western Michigan University, Kalamazoo, MI	B.S.	1972	Mathematics
University of Michigan, Ann Arbor, MI	M.P.H.	1974	Biostatistics
University of Michigan, Ann Arbor, MI	A.M.	1974	Statistics
University of Michigan, Ann Arbor, MI	Ph.D.	1977	Biostatistics

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Key personnel include the principal investigator and any other individuals who participate in the scientific development or execution of the project. Key personnel typically will include all individuals with doctoral or other professional degrees, but in some projects will include individuals at the masters or baccalaureate level provided they contribute in a substantive way to the scientific development or execution of the project. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. DO NOT EXCEED TWO PAGES.

1977-1978 Assistant Professor of Statistics, Department of Mathematics, Montana State University, Bozeman, Montana.

1978-1980 Biostatistician, Domestic Pharmaceutical Medical Affairs, The Upjohn Company, Kalamazoo, Michigan.

1980-1981 Assistant Professorial Lecturer in Statistics, Department of Statistics, George Washington University, Washington, D.C.

1981-1984 Associate Professorial Lecturer in Statistics, Department of Statistics, George Washington University, Washington, D.C.

1980-1989 Statistician, Statistical Resource Section, Field Studies and Biometry Branch, Epidemiology and Biometry Program, Division of Epidemiology and Clinical Applications, National Heart, Lung, and Blood Institute, Bethesda, Maryland.

1989-present Professor and Head, Division of Biostatistics, Department of Medicine, University of Virginia School of Medicine, Charlottesville, Virginia.

ADVISORY COMMITTEE:

Scientific Advisory Committee, United States Renal Data System, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, 1989-present.

SELECTED PUBLICATIONS:

Abbott RD, Garrison RJ, Wilson PW, Castelli WP. Coronary Heart Disease Risk: The Importance of Joint Relationships Among Cholesterol Levels in Individual Lipoprotein Classes. **Preventive Medicine** 11:131-141, 1982.

Kannel WB, Abbott RD, Savage DD, McNamara PM. Epidemiologic Features of Chronic Atrial Fibrillation: The Framingham Study. **New England Journal of Medicine** 306:1018-1022, 1982.

Castelli WP, Abbott RD, McNamara PM. Summary Estimates of Cholesterol Used to Predict Coronary Heart Disease. **Circulation** 67:730-734, 1983.

Abbott RD, Garrison RJ, Wilson PW, Epstein FH, Castelli WP, Feinleib M, LaRue C. Joint Distribution of Lipoprotein Cholesterol Classes: The Framingham Study. **Arteriosclerosis** 3:260-272, 1983.

Kannel WB, Abbott RD. Incidence and Prognosis of Unrecognized Myocardial Infarction: An Update on the Framingham Study. **New England Journal of Medicine** 311:1144-1147, 1984.

Abbott RD, Carroll RJ. Interpreting Multiple Logistic Regression Coefficients in Prospective Observational Studies. **American Journal of Epidemiology** 119:830-836, 1984.

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Halperin M, Abbott RD, Blackwelder WC, Jacobowitz J, Lan KKG, Verter J, Wedel H. On the Use of the Logistic Model in Prospective Studies. **Statistics in Medicine** 4:227-235, 1985.

Brand FN, Abbott RD, Kannel WB, Wolf PA. Characteristics and Prognosis of Lone Atrial Fibrillation: 30-Year Follow-up in the Framingham Study. **Journal of the American Medical Association** 254:3449-3453, 1985.

Abbott RD. Logistic Regression in Survival Analysis. **American Journal of Epidemiology** 121:465-471, 1985.

Abbott RD, Carroll RJ. Conditional Regression Models for Transient State Survival Analysis.. **American Journal of Epidemiology** 123:728-735, 1986.

Donahue RP, Abbott RD, Reed DM, Yano K. Alcohol and Hemorrhagic Stroke: The Honolulu Heart Program. **Journal of the American Medical Association** 255:2311-2314, 1986.

Castelli WP, Garrison RJ, Wilson PWF, Abbott RD, Kalousdian S, Kannel WB. Incidence of Coronary Heart Disease and Lipoprotein Cholesterol Levels: The Framingham Study. **Journal of the American Medical Association** 256:2835-2838, 1986.

Abbott RD, Yin Y, Reed DM, Yano K. Risk of Stroke in Male Cigarette Smokers. **New England Journal of Medicine** 315:717-720, 1986.

Donahue RP, Abbott RD, Bloom E, Reed DM, Yano K. Central Obesity and Coronary Heart Disease in Men. **Lancet** 1:821-824, 1987.

Wolf PA, Abbott RD, Kannel WB. Atrial Fibrillation - A Major Contributor to Stroke in the Elderly: The Framingham Study. **Archives of Internal Medicine** 147:1561-1564, 1987.

Abbott RD, Donahue RP, MacMahon SW, Reed DM, Yano K. Diabetes and the Risk of Stroke: The Honolulu Heart Program. **Journal of the American Medical Association** 257:949-952, 1987.

Abbott RD, Brand FN, Kannel WB, Castelli WP. Gout and Coronary Heart Disease: The Framingham Study. **Journal of Clinical Epidemiology** 41:237-242, 1988.

Abbott RD, Wilson PWF, Kannel WB, Castelli WP. High Density Lipoprotein Cholesterol, Total Cholesterol Screening, and Myocardial Infarction: The Framingham Study. **Arteriosclerosis** 8:207-211, 1988.

Abbott RD, Donahue RP, Kannel WB, Wilson PWF. The Impact of Diabetes on Survival Following Myocardial Infarction in Men Vs Women: The Framingham Study. **Journal of the American Medical Association** 260:3456-3460, 1988.

Brand FN, Abbott RD, Kannel WB. Diabetes, Intermittent Claudication, and the Risk of Cardiovascular Events: The Framingham Study. **Diabetes** 38:504-509, 1989.

Abbott RD, Levy D, Kannel WB, Castelli WP, Wilson PWF, Garrison RJ, Stokes III J. Cardiovascular Risk Factors and Graded Treadmill Exercise Endurance in Healthy Adults: The Framingham Offspring Study. **American Journal of Cardiology** 63:342-346, 1989.

MacMahon S, Peto R, Cutler J, Collins R, Sorlie P, Neaton J, Abbott R, Godwin J, Dyar A, Stamler J. Blood Pressure, Stroke, and Coronary Heart Disease. **Lancet** 335:765-774, 1990.

Abbott RD, Brand FN, Kannel WB. Epidemiology of Some Peripheral Arterial Findings in Diabetic Men and Women: Experiences From the Framingham Study. **American Journal of Medicine** 88:376-381,, 1990.

Reed D, Abbott RD, Smucker ML, Kaul S. Prediction of Outcome Following Mitral Valve Replacement in Patients with Symptomatic Chronic Mitral Regurgitation: The Importance of Left Atrial Size. **Circulation** 84:23-34, 1991.

Sabia P, Abbott RD, Afrookteh A, Touchstone DA, Kaul S. The Importance of Two-Dimensional Echocardiographic Assessment of Left Ventricular Systolic Function in Patients Presenting to the Emergency Room with Cardiac Related Symptoms. **Circulation** 84:1615-1624, 1991

Pollock SG, Abbott RD, Boucher CA, Beller GA, Kaul S. Independent and Incremental Prognostic Value of Tests Performed in Hierarchical Order to Evaluate Patients with Suspected Coronary Artery Disease: Validation of Models Based on These Tests. **Circulation** 85:237-248, 1992.

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Appendix A

IRB FORMS, QUESTIONNAIRES, AND ANIMAL APPROVAL FORMS

A1. IRB form: "Risk Factors for Acute Episodes of Wheezing in Infants and Children"

A2. Enrollment Questionnaire.

A3. Physical Examination Form.

A4. IRB form (final approval pending): "Immune Responses to Inhalant and Food Allergens During Early Childhood"

A5. Home Visit Questionnaire.

A7. University of Virginia - Animal Protocol Certification.

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OMB No. 0925-0637

DEPARTMENT OF HEALTH AND HUMAN SERVICES

PROTECTION OF HUMAN SUBJECTS ASSURANCE/CERTIFICATION/DECLARATION

☐ ORIGINAL ☐ FOLLOWUP ☐ EXEMPTION (previously undesignated)

☐ GRANT ☐ CONTRACT ☐ FELLOW ☐ OTHER

☐ New ☐ Competing continuation ☐ Noncompeting continuation ☐ Supplemental

APPLICATION IDENTIFICATION NO. *(if known)*

POLICY: A research activity involving human subjects that is not exempt from HHS regulations may not be funded unless an Institutional Review Board (IRB) has reviewed and approved the activity in accordance with Section 474 of the Public Health Service Act as implemented by Title 45, Part 46 of the Code of Federal Regulations (45 CFR 46—as revised). The applicant institution must submit certification of IRB approval to HHS unless the applicant institution has designated a specific exemption under Section 46.101(b) which applies to the proposed research activity. Institutions with an assurance of compliance on file with HHS which covers the proposed activity should submit certification of IRB review and approval with each application. (In exceptional cases, certification may be accepted up to 60 days after the receipt date for which the application is submitted.) In the case of institutions which do not have an assurance of compliance on file with HHS covering the proposed activity, certification of IRB review and approval must be submitted within 30 days of the receipt of a written request from HHS for certification.

1. TITLE OF APPLICATION OR ACTIVITY

Risk Factors for Acute Episodes of Wheezing in Infants and Children

2. PRINCIPAL INVESTIGATOR, PROGRAM DIRECTOR, OR FELLOW

Peter W. Heymann, M.D.

3. FOOD AND DRUG ADMINISTRATION REQUIRED INFORMATION *(see reverse side)*

4. HHS ASSURANCE STATUS

☒ This institution has an approved assurance of compliance on file with HHS which covers this activity.

M1343 Assurance identification number 01XB IRB identification number

☐ No assurance of compliance which applies to this activity has been established with HHS but the applicant institution will provide written assurance of compliance and certification of IRB review and approval in accordance with 45 CFR 46 upon request.

5. CERTIFICATION OF IRB REVIEW OR DECLARATION OF EXEMPTION

☒ This activity has been reviewed and approved by an IRB in accordance with the requirements of 45 CFR 46, including its relevant Subparts. This certification fulfills, when applicable, requirements for certifying FDA status for each investigational new drug or device *(see reverse side of this form)*.

_____ Date of IRB review and approval. *(If approval is pending, write "pending". Followup certification is required.)*
(month/day/year)

☐ Full Board Review ☒ Expedited Review

for continuation of previously approved protocol

☐ This activity contains multiple projects, some of which have not been reviewed. The IRB has granted approval on condition that all projects covered by 45 CFR 46 will be reviewed and approved before they are initiated and that appropriate further certification *(form HHS 596)* will be submitted.

☐ Human subjects are involved but this activity qualifies for exemption under 46.101(b) in accordance with paragraph _____ *(insert paragraph number of exemption in 46.101(b), 1 through 6)*, but the institution did not designate that exemption on the application.

6. Each official signing below certifies that the information provided on this form is correct and that each institution assumes responsibility for assuring required future reviews, approvals, and submissions of certification.

APPLICANT INSTITUTION	COOPERATING INSTITUTION
NAME, ADDRESS, AND TELEPHONE NO. University of Virginia Health Sciences Ctr Charlottesville VA 22908 (804) 924-2109	NAME, ADDRESS, AND TELEPHONE NO.
NAME AND TITLE OF OFFICIAL *(print or type)* John A. Owen, Jr., M.D., Chair Human Investigation Committee	NAME AND TITLE OF OFFICIAL *(print or type)*
SIGNATURE OF OFFICIAL LISTED ABOVE *(and date)* John A. Owen, Jr. 6/24/93	SIGNATURE OF OFFICIAL LISTED ABOVE *(and date)*

HHS 596 (Rev. 1/82) *(If additional space is needed, please use reverse side under "Notes.")*

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Group # _____

PEDIATRIC WHEEZING STUDY - PATIENT QUESTIONNAIRE

Patient's Name _____ History # _____

Phone (home) _____ (work) _____

Address _____

Date of ER visit _____ Date of birth _____ Age _____

Sex: M F Race: B W Other _____

Relationship of person interviewed to patient _____

1) **Was your child born at UVA?** Yes No 2) **Birth weight:** _____

3) **Was he/she premature?** Yes No 4) **Did he/she need O_2 after birth?** Yes No

5) **How long was he/she hospitalized?** _____

6) **Was your child breast fed? (circle one)** 2 months 2-4 months 4-6 months >6 months currently? unknown

7) **Family or personal history for CF?**

8) **Personal history for: cardiac or pulmonary abnormalities?** Yes No
foreign body? Yes No

9) **Which family members have or have had problems with:**

	Mother			Father			Siblings		
Asthma	yes	no	unknown	yes	no	unknown	yes	no	unknown
Hay Fever	yes	no	unknown	yes	no	unknown	yes	no	unknown
Eczema	yes	no	unknown	yes	no	unknown	yes	no	unknown
Food Allergy (specify)	yes	no	unknown	yes	no	unknown	yes	no	unknown
Any Other Documented Allergies	yes	no	unknown	yes	no	unknown	yes	no	unknown

10) **Has your child ever had problems with (circle all that apply):**
a) eczema (atopic dermatitis): As an infant? Yes No **Currently?** Yes No
b) hay fever (allergic rhinitis)? Yes No
c) foods which make your child sick? Yes No **Describe** _____

_____

11) **Where does your child receive most of his/her medical care? (circle)**
a) Emergency Room
b) Clinic (Primary Care Center)
c) Private Doctor's Office
d) Prompt Care Centers (HMO)
e) Health Department
f) Other _____

12) **Has your child ever had problems with wheezing prior to this visit?** Yes No
At what age did this start? _____ **How many ER visits for wheezing?** _____
How many hospitalizations for wheezing? _____
When did this recent attack start? _____
When was your child last seen for wheezing? _____

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

13) **Does your child take medicines for asthma?**

	PRN	Daily	Currently (How much?)
Beta agonist, inhaler	___	___	_____
Beta$_2$-agonist, p.o.	___	___	_____
Beta$_2$-agonist, inhaler	___	___	_____
Steroid, p.o.	___	___	_____
Steroid, inhaler	___	___	_____
Theophylline	___	___	_____
Cromolyn	___	___	_____
Other	___	___	_____

14) **Who spends most time taking care of your child?** _____

15) **Does your child attend day care, nursery school, grade school? (circle one)**

16) **Does anyone smoke at day care or nursery school?** Yes No Not known

17) **How many people are living at home?** Adults _____ (18 years or older)
Children _____ (less than 18 years)

18) **How many people smoke at home?** _____ (Mark an "X" under each that applies.)

	Mother	Father	Other _____	Other _____	Patient
a) ≤ 5 cigarettes/day					
b) ≥ ½ pack/day					
c) cigars or pipe regularly					
d) smoking location					

19) **Parent education level?** _____ (mother) _____ (father)

20) **In what kind of dwelling do you live? (Circle one.)**
- **a) house**
- **b) townhouse/duplex**
- **c) apartment (basement, ground floor, other)**
- **d) trailer**
- **e) other** _____

21) **How is your home heated? (Circle all that apply.)**
- **a) wood stove**
- **b) kerosene heater**
- **c) electric baseboard**
- **d) heat pump**
- **e) other (oil or gas) and comment on forced air through vents** _____

22)

Exposure to animals?	**Inside**	**Outside**
Cat	_____	_____
Dog	_____	_____
Other _____	_____	_____

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

Group # _____, 10/92

Pediatric Wheezing Study - Physical Assessment

Patient Name_____ History #_____

Date of visit_____

1) **Patient's signs and symptoms:** (Please circle those which apply):

Nasal congestion/rhinorrhea	Wheezing	Eczema
Conjunctivitis	Retractions	Viral exanthem
Fever	Cough	Rales
Other_____		

Physical Parameters	(Circle appropriate findings.)		
Expiratory wheeze	None	Audible only with stethoscope	Audible without stethoscope
Retractions	None	Mild	Marked
Inspiratory Breath Sounds	Normal	Decreased	Markedly decreased or absent
Cyanosis	None	In room air	In supp. O_2
Consciousness	Normal	Depressed or agitated	Unconscious

2) **Medications prescribed for:**

	ER management	Discharge medications
$Beta_2$-agonist, p.o.	____	____
$Beta_2$-agonist, inhaler	____	____
Steroid, p.o.	____	____
Steroid, inhaler	____	____
Theophylline	____	____
Cromolyn	____	____
Other	____	____

3) **Was patient admitted?** YES NO

4) **Discharge diagnosis:** _____

_____

5) **Comments:**

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

OMB No. 0925-0637

DEPARTMENT OF HEALTH AND HUMAN SERVICES

PROTECTION OF HUMAN SUBJECTS ASSURANCE/CERTIFICATION/DECLARATION

☐ ORIGINAL ☐ FOLLOWUP ☐ EXEMPTION (previously undesignated)

☐ GRANT ☐ CONTRACT ☐ FELLOW ☐ OTHER

☐ New ☐ Competing continuation ☐ Noncompeting continuation ☐ Supplemental

APPLICATION IDENTIFICATION NO. *(if known)*

POLICY: A research activity involving human subjects that is not exempt from HHS regulations may not be funded unless an Institutional Review Board (IRB) has reviewed and approved the activity in accordance with Section 474 of the Public Health Service Act as implemented by Title 45, Part 46 of the Code of Federal Regulations (45 CFR 46—as revised). The applicant institution must submit certification of IRB approval to HHS unless the applicant institution has designated a specific exemption under Section 46.101(b) which applies to the proposed research activity. Institutions with an assurance of compliance on file with HHS which covers the proposed activity should submit certification of IRB review and approval with each application. (In exceptional cases, certification may be accepted up to 60 days after the receipt date for which the application is submitted.) In the case of institutions which do not have an assurance of compliance on file with HHS covering the proposed activity, certification of IRB review and approval must be submitted within 30 days of the receipt of a written request from HHS for certification.

1. TITLE OF APPLICATION OR ACTIVITY

Immune Responses to Inhalant and Food Allergens During Early Childhood

2. PRINCIPAL INVESTIGATOR, PROGRAM DIRECTOR, OR FELLOW

Peter W. Heymann, M.D.

3. FOOD AND DRUG ADMINISTRATION REQUIRED INFORMATION *(see reverse side)*

4. HHS ASSURANCE STATUS

☒ This institution has an approved assurance of compliance on file with HHS which covers this activity.

M1343 Assurance identification number 01XB IRB identification number

☐ No assurance of compliance which applies to this activity has been established with HHS but the applicant institution will provide written assurance of compliance and certification of IRB review and approval in accordance with 45 CFR 46 upon request.

5. CERTIFICATION OF IRB REVIEW OR DECLARATION OF EXEMPTION

☒ This activity has been reviewed and approved by an IRB in accordance with the requirements of 45 CFR 46, including its relevant Subparts. This certification fulfills, when applicable, requirements for certifying FDA status for each investigational new drug or device *(see reverse side of this form)*.

_____ Date of IRB review and approval. *(If approval is pending, write "pending". Followup certification is required.)*
(month/day/year)

☐ Full Board Review ☒ Expedited Review

for continuation of previously approved protocol

☐ This activity contains multiple projects, some of which have not been reviewed. The IRB has granted approval on condition that all projects covered by 45 CFR 46 will be reviewed and approved before they are initiated and that appropriate further certification *(form HHS 596)* will be submitted.

☐ Human subjects are involved but this activity qualifies for exemption under 46.101(b) in accordance with paragraph _____ *(insert paragraph number of exemption in 46.101(b), 1 through 5)*, but the institution did not designate that exemption on the application.

6. Each official signing below certifies that the information provided on this form is correct and that each institution assumes responsibility for assuring required future reviews, approvals, and submissions of certification.

APPLICANT INSTITUTION	COOPERATING INSTITUTION
NAME, ADDRESS, AND TELEPHONE NO. University of Virginia Health Sciences Ctr Charlottesville VA 22908 (804) 924-2109	NAME, ADDRESS, AND TELEPHONE NO.
NAME AND TITLE OF OFFICIAL *(print or type)* John A. Owen, Jr., M.D., Chair Human Investigation Committee	NAME AND TITLE OF OFFICIAL *(print or type)*
SIGNATURE OF OFFICIAL LISTED ABOVE *(and date)* John A. Owen, Jr. 6/24/93	SIGNATURE OF OFFICIAL LISTED ABOVE *(and date)*

HHS 596 (Rev. 1/82)

(If additional space is needed, please use reverse side under "Notes.")

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

PEDIATRIC ALLERGY STUDY
Home Visit Questionnaire

Interviewer _____ Date _____

Patient _____

Relationship of interviewee to patient _____ Phone # _____

Address _____

_____

1) Type of home: House Town House Apartment Trailer
2) Approximate age of home _____ 3) No. of bedrooms _____
4) How long has family lived there? _____ 5) No. of people living and sleeping at home _____
6) Is there a basement? _____ Carpeted? _____
7) On what floor is patient's bedroom? _____ Carpeted? _____
8) Type of heating? _____
9) Is there air conditioning? _____ &/or humidifier? _____
10) Are there pets? _____
11) Where are they kept? _____
12) Do pets sleep in the patient's bedroom? _____
13) Who smokes at home and how much? _____

_____

14) Where does patient spend most of the day?
Home Day care Another home (sitter, etc.) School
15) If patient spends most of the day at day care or another home:
Do people smoke there? (quantify) _____
Approximately how many children are present there? _____
16) Has the family undertaken any allergen avoidance measures prior to this visit?

_____

17) Family income assessment: <$10,000 ___ $30-40,000 ___
$10-20,000 ___ $40-50,000 ___
$20-30,000 ___ >$50,000 ___
Other _____
How many family members does this income support? _____
18) Health insurance status: No insurance _____ Medicaid _____
Other _____

19) Education background:

	Mother	Father	Guardian
Grade completed in high school:	_____	_____	_____
College education (Yes/No):	_____	_____	_____
Post-graduate education (Yes/No):	_____	_____	_____

20) What samples (dust, saliva, blood etc.) were collected at today's visit?

_____

21. Other comments: _____

_____

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

UNIVERSITY OF VIRGINIA PROTOCOL CERTIFICATION
FOR GRANT APPLICATIONS

* * * \

Appendix B

LETTERS OF SUPPORT

1) Martin D. Chapman, Ph.D.	Assistant Professor of Medicine and Microbiology, University of Virginia, Department of Medicine
2) Barbara B. Wilson, M.D.	Assistant Professor, Dermatology Head, Immunodermatology Clinic University of Virginia
3) William M. Sutherland, Ph.D.	Research Associate Professor of Anatomy and Cell Biology, Associate Director, Lymphocyte Culture Center, University of Virginia
4) Gregory F. Hayden, M.D.,	Professor of Pediatrics, Division Head, General Pediatrics University of Virginia
5) Richard A. Christoph, M.D.	Assistant Professor, Medicine and Pediatrics Director, Pediatric Emergency Service University of Virginia
6) Robert D. Abbott, Ph.D.	Professor and Head, Division of Biostatistics University of Virginia
7) R. Ariel Gomez, M.D.	Associate Professor of Pediatrics Chief, Pediatric Nephrology Program Director, CHRC University of Virginia.

2028388183

Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

UNIVERSITY OF VIRGINIA
HEALTH SCIENCES CENTER
DEPARTMENT OF MEDICINE

June 22, 1993

Peter W. Heymann, M.D.
Pediatrics Department
MR-4, 2006

Dear Peter,

I am pleased to support your NIH grant application to work on the role of inhaled allergens and food allergens in the etiology of asthma and other allergic diseases in children. As far as inhaled allergens are concerned, I am keen to continue our collaborative studies and to build upon our recent success in developing an ELISA for Group II allergens of *D. pteronyssinus* and *D. farinae*. This new assay should be particularly useful for further assessment of risk levels for sensitization and exposure to mite allergens and should also be useful for assessing the efficacy of avoidance regimes.

In addition to these studies, we will be pleased to provide you with the monoclonal immunoassays for cat allergen (*Fel d* I) and for the two German cockroach allergens (*Bla g* I and *Bla g* II). These assays have been used to assess both cat and cockroach allergen exposure (see De Blay, *et al.*, Am Rev Respir Dis 1991, 143:1334-1339; and Pollart *et al.*, JACI 1991, 87:505-21) and are currently being used in the NIH Cooperative Study on Inner City Asthma (NCICAS). We have highly purified *Fel d* I and cockroach allergens, which can be used in antigen binding immunoassays to measure IgG and IgE antibody responses in the children enrolled in your study. We would be particularly interested in comparing the responses to cockroach allergens in these children, since at present most of our data on sensitization to the different allergens is based on adult studies.

I strongly support your application and hope that it is successful. Please let me know if I can be of any further help.

Best wishes,

Yours sincerely,

Mark Chapman

Martin D. Chapman, Ph.D.
Associate Professor of Medicine & Microbiology

DIVISION OF ALLERGY AND CLINICAL IMMUNOLOGY
HEALTH SCIENCES CENTER BOX 225, CHARLOTTESVILLE, VIRGINIA 22908 804-924-2227

2028388184

Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

UNIVERSITY OF VIRGINIA
HEALTH SCIENCES CENTER
DERMATOLOGY DEPARTMENT

June 22, 1993

PEYTON E. WEARY, M.D.
CHAIRMAN
KENNETH E. GREER, M.D.
HARRY L. PARLETTE III, M.D.
BARBARA B. WILSON, M.D.

Dr. Peter Heyman
Building MR4, Room 5061
Box 29
UVA Medical Center

Dear Peter:

I am writing to let you know that I am very interested in supporting your study of the development of inhalant allergy in infants and young children with atopic dermatitis. It will be great to be able to follow them and pinpoint when it is they develop inhalant allergies, and especially allergies to dust mites. As you know, we see a number of atopic children, both in our Dermatology clinic and in our Immunodermatology clinic, and don't think that we should have trouble finding appropriate participants for the study.

I look forward to working with you.

Sincerely yours,

Barbara B. Wilson, M.D.
Associate Professor of
Dermatology

UNIVERSITY HOSPITALS BOX 134
CHARLOTTESVILLE, VIRGINIA 22908
804-924-5115
FAX 804-982-1841
804-982-HEAR V/TDD

2028388185

Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

UNIVERSITY OF VIRGINIA
HEALTH SCIENCES CENTER
LYMPHOCYTE CULTURE CENTER

June 23, 1993

Dr. Peter Heymann
MR 4, Box 29
Health Sciences Center

Dear Peter:

The Lymphocyte Culture Center (LCC) is a research support facility of the University of Virginia School of Medicine. The primary function of the LCC is to make available to researchers the most current technology and expertise for the construction of lymphocyte-myeloma hybrids for the production of monoclonal antibodies. The LCC can also provide expertise in the use of these reagents in basic research programs.

The center occupies an approximately 510 square foot modern laboratory equipped with cell culture incubators, laminar flow hoods, centrifuges, inverted and standard microscopes, automated microplate washer and ELISA plate reader, etc. We also have facilities for the cryopreservation of established hybrids and myeloma cell lines in either liquid nitrogen or at -80°C. The LCC is currently staffed by myself, a laboratory specialist C, a laboratory specialist A and a part-time laboratory aide.

During the past ten years of operation, the LCC has successfully completed a number of projects for investigators throughout the University and the Medical School. We have also successfully completed a contract with the Federal Bureau of Investigation involving the production and characterization of monoclonal antibodies for forensic applications. The hybridomas secreting these antibodies have been patented and kits employing these reagents for forensic diagnosis are commercially available. We routinely obtain fusion frequencies greater than 85% (wells containing viable hybrids/wells plated x 100) or approximately one in every 2×10^5 spleenocytes forming a viable hybrid. As a result of these high fusion frequencies, we have been able to construct hybridomas secreting monoclonal antibodies specific to the immunogen from a single fusion in most of the projects that we have initiated.

Our standard mouse fusion protocol (M.D. Chapman, W.M. Sutherland, and T.A.E. Platts-Mills (1984), J. Immunol. 133:2488-2495) is modified from published procedures (Oi, V.T. and Herzenberg, L.A. in Selected Methods in Cellular Immunology, ed. B.B. Mishell and S.M. Shiigi, W.H. Freeman & Co., 1980, p. 351). Briefly, spleen cells from an immunized mouse are washed once in Iscove's MDM and mixed with washed Sp2/0-Ag14 myeloma cells (Shulman, et al. (1978), Nature 276:269) at a 5:1 ratio (spleenocyte to myeloma). The cells are pelleted and the medium aspirated. Cell fusion is accomplished by the stepwise addition of 37% polyethylene glycol (PEG 1,000, Koch-Light) over one minute. The PEG is then diluted dropwise with 10 ml Iscove's MDM and the cells pelleted and gently washed once in Iscove's MDM containing 15% selected fetal bovine serum, hypoxanthine (H), and thymidine (T). The cells are resuspended in this HT medium, transferred to a petri dish and incubated at 37°C in a humidified

HEALTH SCIENCES CENTER BOX 439 CHARLOTTESVILLE, VIRGINIA 22908 804-924-1913 804-982-HEAR V/TDD FAX 804-982-3912

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

atmosphere of 5% CO_2/95% air for one hour. The cells are then resuspended in HT medium and plated into 96-well tissue culture plates (Costar, Cambridge, Mass.) at a density of approximately 2-4 x 10^5 cells/well. The cultures are fed 24 hours later with HT medium containing aminopterin (HAT medium) and maintained in this medium for two weeks. Macroscopic colonies usually appear within 7-10 days following the fusion and supernatants are screened for specific antibody production. Positive hybrids are cloned twice by limiting dilution, and frozen cell stocks are stored in liquid nitrogen cell banks. We also routinely generate bulk monoclonal antibody as either ascites, or as culture supernatants in either fetal bovine serum containing medium or in a defined medium (protein free). The LCC also provides the routine purification of monoclonal antibodies by affinity chromatography on engineered recombinant Protein G columns.

In addition to providing all of the cell culture aspects of hybridoma production, we can also assist in developing assay procedures tailored to your specific needs. Enzyme linked immunosorbent assays (ELISA) [Engvall, E. and Perlman, P., "Enzyme linked immunosorbent assay (ELISA): quantitative assay for immunoglobin." Immunochem. 8:871-874 (1971)] are routinely performed in this laboratory for antigen specific antibody production, antibody class and subclass identification, and non-specific immunoglobulin production and quantitation [Engvall, E. and Pesce, A.J., eds. Quantitative Enzyme Immunoassay, Scad. J. Immunol. (Suppl. &) 1978]. These assays may be either qualitative or quantitative (using our Titertek Multiskan MC microplate reader, Flow Laboratories) and form an integral part of the services provided by the LCC.

Please contact me if I can be of further assistance to you in meeting the goals of your research projects.

Sincerely,

Bill

William M. Sutherland, Ph.D.
Associate Director
Lymphocyte Culture Center
Research Associate Professor of
Anatomy and Cell Biology

Phone: 924-5379

Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

2028388187

UNIVERSITY OF VIRGINIA
HEALTH SCIENCES CENTER
CHILDREN'S MEDICAL CENTER
DEPARTMENT OF PEDIATRICS

June 24, 1993

Peter Heymann, M.D.
Division of Pediatric Allergy
Department of Pediatrics
Bldg. MR-4; Room 5061; Box 29
University of Virginia Health Sciences Center

Dear Peter,

I am happy to learn about your grant proposal to continue further studies on the development of allergic manifestations in infants and young children. This sounds like a terrific project with important clinical ramifications. I would anticipate great interest in this study among staff and families in both the General Pediatric Clinic in the Primary Care Center and in our Northridge Pediatric Practice. I will be happy to provide any support I can in terms of patient recruiting at both of these study sites. Please let me know how I can be of assistance.

Sincerely yours,

Gregory F. Hayden, M.D.
Professor of Pediatrics
Division Head, General Pediatrics
University of Virginia Health
Sciences Center

GFH/bsr

BOX 501 CHARLOTTESVILLE, VIRGINIA 22908 804-924-2250

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

UNIVERSITY OF VIRGINIA
HEALTH SCIENCES CENTER
EMERGENCY MEDICAL SERVICES

June 22, 1993

Peter Heymann, MD
Associate Professor of Pediatrics
Chief, Division of Pediatric Allergy
University of Virginia Health
Sciences Center

Dear Dr. Heymann:

The Pediatric Emergency Service at the University of Virginia is delighted to indicate its strong support for your study regarding the development for your study regarding the development of IgE antibody to inhaled allergens in relation to viral respiratory tract infections and tobacco smoke exposure in wheezing children.

As you know, the management of children with asthma and bronchiolitis is a very common problem in the emergency department setting. We believe that studies clarifying the etiology of these episodes of reactive airway disease will only enhance the management of these children. The insights gained through your previous studies on this subject have greatly expanded and deepened our understanding of the pathophysiology of wheezing in children.

We are easily pleased with the commitment demonstrated by your staff to the enrolling of patients for your studies in the emergency department. The attending faculty, the nursing staff, and the houseofficers in the Pediatric Emergency Service all continue to be highly supportive of your efforts in the recruitment of patients. We have found that enrolling patients for your previous studies has resulted in very little inconvenience to patient flow in the emergency department and has been generally well received by prospective enrollees. We anticipate little difficulty in continuing the project in the emergency department and remain open to facilitating your program in any way possible.

Best regards,

Richard A. Christoph MD

Richard A. Christoph, MD
Associate Professor of Pediatrics and
Internal Medicine
Director, Pediatric Emergency Service
University of Virginia Health
Sciences Center

(RCJUN93.22)

UNIVERSITY HOSPITALS, BOX 523, CHARLOTTESVILLE, VIRGINIA 22908 804-924-8485

2028388189

Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

UNIVERSITY OF VIRGINIA
HEALTH SCIENCES CENTER
DEPARTMENT OF MEDICINE

Division of Biostatistics

Robert D. Abbott, Ph.D.
Professor and Head

Box 432
Health Sciences Center
Charlottesville, Virginia 22908
804-924-1687
FAX: (804) 924-5439

June 25, 1993

Peter Heymann, M.D.
Department of Pediatrics
MR4, Box 29, Room 5061

Dear Peter,

I would be delighted to provide you with biostatistical support in your investigation into the "development of inhalant allergy and asthma in children". While participating in your project in all aspects of statistical modeling, analysis, and data interpretation, I also offer you the full support of the Division of Biostatistics. I look forward to working with you on this very exciting project.

Sincerely yours,

Robert D. Abbott

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

UNIVERSITY OF VIRGINIA
HEALTH SCIENCES CENTER
CHILDREN'S MEDICAL CENTER
DEPARTMENT OF PEDIATRICS

June 21, 1993

Peter Heymann, M.D.
Associate Professor of Pediatrics
Chief, Allergy Division
MR4/2017

Dear Peter:

I am very excited about your studies on the development of inhalant allergy and asthma in young children. As you know, my laboratory as well as the CHRC Molecular Core Lab have been performing in situ hybridization and immunocytochemistry for the detection of renin, angiotensinogen, AT1 receptor, and other mRNAs in tissue sections and isolated cells. We will be happy to help you with adapting these techniques to the detection of RSV, rhinovirus RNAs, tryptase and ECP. My laboratory as well as the molecular core laboratory will be completely at your disposal for these purposes. In addition, we are currently utilizing in situ PCR for the detection of rare transcripts. If the need arises we will also be happy to help you set up this technique.

Sincerely,

R. Ariel Gomez, M.D.
Associate Professor of Pediatrics
Chief, Pediatric Nephrology
Program Director, CHRC

RAG/bb

CHARLOTTESVILLE, VIRGINIA 22908

2028388191

Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

Appendix C

PUBLICATIONS

C1. Duff AL, Pomeranz E, Gelber LE, Price GW, Farris AH, Christoph RA, Hayden FG, Platts-Mills TAE, Heymann PW: Risk factors for acute wheezing in infants and children: viruses, passive smoke, and IgE antibodies to inhalant allergens. Pediatrics. In press.

C2. El-Dahr JM, Fink R, Selden R, Arruda LK, Platts-Mills TAE, Heymann PW: Development of immune responses to Aspergillus at an early age in children with cystic fibrosis. American Review of Respiratory Disease. In press.

C3. Duff AL, Wilson BB, Deuell BL, Platts-Mills TAE, Heymann PW: Early childhood inhalant allergen sensitization in atopic eczema. Submitted.

C4. Ovsyannikova IG, Vailes LD, Ying L, Heymann PW, Chapman MD: Monoclonal antibodies to Group II *Dermatophagoides spp.* allergens: Murine immune response, epitope analysis and development of a two site ELISA. Submitted.

C5. Price GW, Duff AL, Farris AH, Burks AW, Platts-Mills TAE, Heymann PW: Sensitization (IgE antibody) to food allergens in wheezing infants and children. Submitted.

2028388192

Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

Risk Factors for Acute Wheezing in Infants and Children: Viruses, Passive Smoke, and IgE Antibodies to Inhalant Allergens

Angela L. Duff, M.D.[‡*], Elaine S. Pomeranz, M.D.[§], Lawrence E. Gelber, M.D.[*], G. Weyman Price, M.D.[*], Holly Farris, B.A.[*], Frederick G. Hayden, M.D.[‡], Thomas A.E. Platts-Mills, M.D., Ph.D.[‡], Peter W. Heymann, M.D.[*]

From:

* Department of Pediatrics, University of Virginia Health Sciences Center

‡ Department of Internal Medicine, University of Virginia Health Sciences Center

§ Department of Pediatrics, University of Michigan Medical Center

Funding Support:

This work was supported by National Institutes of Health Grants AI-24261 and AI-20565. Additional support was provided by the University of Virginia Children's Medical Center.

Address correspondence and reprint requests to:

Peter W. Heymann, M.D.

Division of Allergy and Clinical Immunology

Building MR-4, Room 5061, University of Virginia Health Sciences Center

Charlottesville, Virginia 22908 USA Tel: (804) 924-0408 Fax: (804) 924-5779

2028388193

Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

KEYWORDS: asthma, bronchiolitis, inhalant allergy, viral respiratory tract infection, environmental tobacco smoke

ABBREVIATIONS:

- RAST, radioallergosorbent test
- RSV, respiratory syncytial virus
- GM, geometric mean
- CI, confidence interval

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

ABSTRACT

Objective: To examine the prevalence of viral infection, passive smoke exposure, and IgE antibody to inhaled allergens in infants and children treated for acute wheezing.

Design: Case-control study of actively wheezing children who were compared to children without respiratory tract symptoms.

Setting: University of Virginia Pediatric Emergency Room.

Patients: Convenience sample of 99 wheezing patients (2 months to 16 years) and 57 control patients (6 months to 16 years).

Measurements and Results: Serum IgE antibody to inhalant allergens, measured by RAST, was uncommon in wheezing and control patients under age 2. After age 2, the percentage of RAST positive patients increased markedly and was significantly higher in wheezing patients than controls after age four (72%, n=54, and 30%, n=40, respectively, $p<0.001$). Total IgE levels and nasal eosinophilia were strongly correlated with a positive RAST after age 2. Viral pathogens, predominantly RSV, were identified in nasal washes from 70% (n=20) of wheezing patients under age 2 compared to 20% of controls (n=10), $p<0.05$. After age 2, viruses, particularly rhinovirus, were isolated in washes from 31% (n=70) of wheezing patients, 64% of whom were also RAST positive. Cotinine, a nicotine metabolite, was elevated (≥10ng/ml) in saliva from a large percentage of smoke exposed wheezing patients under age 2 (74%, n=19) compared to those over age 2 (14%, n=51), $p<0.001$. Odds rations for wheezing were significant for virus (8.2, CI=1.3-5.0), and cotinine (4.7, CI=1.0-21.3) in children under 2, and IgE antibody by RAST (4.5, CI=2.0-10.2), virus (3.7, CI=1.3-10.6) and the combination of IgE antibody and virus (10.8, CI=1.9-59.0) were significant risk factors over age 2.

Conclusion: Wheezing children under age 2 had a high rate of viral infection and a low rate of IgE antibody to inhalant allergens. When these children were exposed to

14 of 19

70 of 51

NON SIGN: BROAD RANGE

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

passive smoke, salivary cotinine levels were elevated suggesting heavy exposure. After age 2, sensitization to inhaled allergens became increasingly important and viruses remained a significant risk factor for wheezing. These data support recommendations to reduce tobacco smoke exposure at home, especially for young patients, and to consider sensitization to inhaled allergens and allergen avoidance in wheezing children at an early age, particularly after age 2.

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INTRODUCTION

Over the past decade, mortality due to asthma in children has increased (1). Hospitalization rates for wheezing prior to age 17 have risen by 4.5% per year with children under age four contributing disproportionately to this statistic (2). These changes have occurred despite our expanding knowledge about the pathophysiology of asthma and emphasis on the usage of anti-inflammatory medications (3). Although some patients experience persistent wheezing, many children have acute wheezing episodes followed by periods of remission. Contributing to the increasing morbidity associated with asthma, environmental exposures to air pollution (4), the overuse of inhaled beta-agonists (5), inadequate anti-inflammatory medications (6), and poor access to health care for medically indigent patients have been cited as causes (7). In addition, exposure to inhaled allergens, viral infections and passive tobacco smoke exposure have been correlated with airway hyper-reactivity in a number of studies (8-10).

In infants, wheezing is often diagnosed as bronchiolitis, and a strong association between viral respiratory tract infections (particularly RSV) and wheezing episodes has been shown (11). Although the prevalence of sensitization (i.e. IgE antibody) to inhalant allergens is not clear among infants who wheeze, prospective studies of infants born to allergic families found that IgE antibody to these allergens was not commonly detected before age two (12, 13). In older children, viral pathogens continue to be a risk factor for wheezing (9). In addition, sensitization to inhaled allergens, particularly to dust mite allergen, has been demonstrated in up to 60-80% of school-aged children with asthma (14-15). Passive tobacco smoke exposure has been shown to increase airway hyperresponsiveness in young infants (16), is related to persistent wheezing in grade school asthmatics (17), and is a more common exposure among children from lower socio-economic backgrounds (18).

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The purpose of the present study was to examine the relationship of sensitization to inhaled allergens, viral infection, and passive smoke exposure in a non-selected population of infants and children treated for wheezing in a pediatric emergency room. An additional objective was to determine at what age IgE antibody to inhalant allergens in these children can be demonstrated as a risk factor for wheezing.

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METHODS

Study Population

Study participants included 99 children presenting to the University of Virginia Pediatric Emergency Room from September 1988 through March 1992 with the diagnosis of bronchiolitis or asthma. Entering wheezing patients were from 2 months to 16 years of age, had wheezing documented by auscultation, required treatment with at least a beta-agonist, and had no history suggestive of bronchopulmonary dysplasia. No patient enrolled in the study had taken systemic steroids prior to his/her emergency room visit. Fifty-seven control patients were enrolled in the same setting and were excluded if they had respiratory symptoms at the time of presentation or a previous history of wheezing.

Additional information was obtained from family members by questionnaire and included questions regarding immediate family history (in parents and siblings) for allergic disease, birth history, breast feeding, patient's allergic history and home tobacco smoke exposure. This study was approved by the human investigation committee at the University of Virginia and informed written consent was obtained from parents and/or subjects.

The University of Virginia is a regional medical center for Central Virginia with approximately 400 pediatric emergency room visits for wheezing annually. Most wheezing children under age 2 present to this emergency room from November through March, whereas older asthmatics present more frequently in the spring and fall. In the present study, the majority of patients (94%) were enrolled during the months of September through June. Seventy-eight percent of wheezing children under age two were enrolled from November through February and 74% of the wheezing patients over age 2 were enrolled over a similar time period (October through February). Seventeen percent of all wheezing patients were admitted to the hospital for further management.

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Additional demographic characteristics of the study population are presented in Table 1.

Immunoassays

Serum from patients was obtained by venipuncture and stored at -20°C until analyzed for IgE antibody to inhalant allergens and for total IgE. Allergen specific IgE antibody was measured by quantitative RAST using cyanogen bromide activated filter paper discs coated with extracts from Miles Inc., Hollister-Stier Division, Spokane, WA, as previously described (8). Briefly, the allergen extracts used included dust mite (*D. pteronyssinus* and *D. farinae* tested separately), cat epithelium, short ragweed pollen, rye grass pollen, and mixed cockroach containing allergens from German, American, and Oriental species. Discs coated with extracts were then incubated with patient's serum (diluted 1/4 and 1/16) and IgE antibody bound to allergen was detected using radiolabelled goat anti-human IgE. Using *D.Farinae* coated discs, a reference control curve was established for each assay with serial two-fold dilutions of pooled serum containing 2500 RAST units/ml of IgE antibody to *D.Farinae*. Three negative and three positive control sera were run with each assay. Sera containing ≥40 RAST units/ml to an allergen (equivalent to ~4 ng/ml of allergen specific IgE antibody) were considered positive (8). Total serum IgE was measured using a monoclonal antibody based radioimmunoassay described previously (8).

Virology

For young children (e.g. less than age 5) approximately one ml of normal saline was instilled into each nostril and secretions aspirated using a Tym-Tap disposable aspirator (Xomed Products, Inc., Jacksonville, FL). Approximately two ml of saline were instilled into the nares of older patients as they held their breath, after which they blew their retained secretions into a sterile plastic cup. The secretions were then transferred to

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vials containing one ml of a veal infusion broth (pH 7.4) containing vancomycin (20 μg/ml), gentamicin (50 μg/ml) and amphotericin B (1 μg/ml). For the detection of RSV, cell pellets were processed within 4 hours, and a commercially prepared direct fluorescent antibody assay was used to stain for RSV antigen (Ortho Diagnostic Systems, Inc., Raritan, NJ). Duplicate monolayers of primary rhesus monkey kidney, human embryonic lung fibroblast (WI-38 strain), A-549, and Ohio HeLa cell lines were inoculated with 0.2 ml volumes of sample from each patient to isolate parainfluenza (types 1,2,3, and 4) virus, RSV, rhinovirus, adenovirus, influenza A and B viruses, and enterovirus using standard techniques.

Nasal Smears for Eosinophils

After obtaining nasal washes, a cotton swab was used to transfer excess mucoid secretions from beneath the inferior turbinate of the nose to a glass slide. When dry, secretions were treated with Hansel's stain (Lide Laboratories, Florissant, MD) and each slide was examined under oil immersion by three individuals who recorded the total number of eosinophils present per 100 cells counted on three or more fields. Using mean values recorded by the three examiners, nasal smears containing greater than 10 eosinophils/100 cells counted were regarded as positive and indicative of at least moderate eosinophilia as judged by a scale proposed by Lim (19).

Tobacco Smoke Exposure Measurements

In addition to obtaining information about the number of smokers in the home, saliva samples were collected from each patient. The samples were centrifuged to remove mucin and the supernatants were stored at -20°C. Cotinine, a principal metabolic derivative of nicotine, was measured using an inhibition radioimmunoassay previously described (20). This assay is sensitive for cotinine levels $\geq$1.0 ng/ml. An excellent correlation between serum and salivary cotinine determinations has been reported using

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this assay, and others have reported a close relationship between saliva, serum, and urine measurements in distinguishing active from non-active smokers (21, 22).

Statistical Analysis

To compare wheezing patients and controls, logistic regression models were derived and the presence or absence of wheezing was used as the dependent variable (23). Other variables, such as the presence or absence of virus or IgE antibody by RAST, were used as independent variables. A significant regression coefficient associated with one of the latter variables indicates that the variable is able to discriminate between wheezing patients and controls. Regression coefficients from these models were used to estimate odds ratios and 95% confidence intervals. When samples were small we relied on Fisher's exact test (23). Two sample t-tests were used to compare continuous variables between wheezing patients and controls in the different age groups. All tests of significance were two sided.

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RESULTS

IgE Antibody and Eosinophils

Of the twenty-three patients under age 2 who presented with wheezing, only two (9%) had specific IgE antibody to one or more of the inhalant allergens tested (i.e. were RAST positive) (Figure 1A). Both of these patients were 22 months old and had IgE antibody to dust mite allergen (one also had IgE to rye grass). In this age group, no control patient had IgE antibody to the allergens tested. Only one nasal smear, from one of the 22 month old RAST positive patients, contained greater than 10 eosinophils/100 cells counted (Figure 2). Among 21 wheezing patients between 2 and 4 years of age, sera from 35% were RAST positive compared to 20% from controls (Figure 1A). In this age group, 32% of the wheezing patients, all but one of whom were RAST positive, had positive nasal smears for eosinophils (Figure 2).

Over the age of 4, 72% of the wheezing children were RAST positive compared to 30% of controls, $p < 0.001$ (Figure 1A). Most of the RAST positive patients (86%) had IgE antibody to dust mite allergen (Figure 1B). Nasal smears were positive for eosinophilia in 52% of wheezing patients in this age group, compared to 16% of the controls, $p<0.001$ (Figure 2). Among wheezing patients with a positive nasal smear, 91% were also RAST positive. Among the RAST positive wheezing patients, 64% had a positive smear.

Questionnaire analysis for family history showed a higher prevalence of allergy related symptoms (asthma, rhinitis, and eczema) in immediate family members of wheezing patients, compared to controls older than age two, $p<0.001$ (Table 1). This difference was not apparent prior to age two. Measurements of total serum IgE revealed low levels for both wheezing and control patients under age two (Table 1). Over age two, total IgE levels for wheezing patients differed significantly from controls, $p<0.001$.

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A rise in total serum IgE was evident in wheezing children from 2 to 4 years of age (GM=86 IU/ml; 95% CI, 37 to 196 IU/ml) and, in addition, RAST positive wheezing patients after age two had significantly higher total IgE levels than the RAST negative patients (GM=365 and 67 IU/ml, respectively, $p<0.001$).

Detection of Viruses

Cultures from 70% of nasal washes from wheezing patients under age two were positive for virus (Figure 3). Of these, ten (50%) were positive for RSV, three for rhinovirus, one for influenza B, and one was positive for both RSV and enterovirus. In children over the age of two, 31% of the cultures from washes were positive for virus. Sixteen were positive for rhinovirus, only three for RSV, two for enterovirus, and one for influenza A. Among this virus positive group, 64% (14 of 22 patients) were also found to be RAST positive. However, the percentages of virus positive washes from RAST positive and RAST negative wheezing patients were similar (14/44=32% and 8/24=33%, respectively). In both age groups, significantly fewer washes from control patients were positive for virus (Figure 3).

Smoke Exposure

Seventy-five percent of all wheezing patients and 58% of control patients had one or more smokers present at home as judged by questionnaire. Analysis of cotinine concentrations in saliva revealed high levels in many of the children under the age of two. The geometric mean of cotinine determinations was significantly elevated for wheezing patients compared to controls (9.8 and 4.3 ng/ml, respectively, $p<0.05$); however, fewer control patients were exposed to smoke at home (Fig. 4). After age two, cotinine concentrations in saliva from wheezing and control patients did not differ significantly (GM=3.6 and 3.5 ng/ml, respectively). In previous studies of salivary cotinine, levels ≥10ng/ml have been reported to be indicative of heavy passive smoke

Range?

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exposure (24). Among the smoke exposed patients in this study, cotinine levels ≥10 ng/ml were detected in a significantly larger percentage of wheezing children under age two (74%, n=19) as compared to wheezing children over age two (14%, n=51), p<0.001 (Fig. 4). All wheezing and control patients with cotinine levels ≥10ng/ml (n=27) were noted to be exposed to one or more smokers at home by questionnaire analysis, except one (Fig. 4).

Combined Risk Factors

Sixty-six percent of wheezing patients in the study had evidence for two or more of the risk factors examined compared to 28% of control patients, p<0.001. Under age 2, 67% of wheezing patients had both exposure to tobacco smoke and a positive viral culture. Over age 2, 64% had at least two of the three risk factors. Odds ratios based on logistic regression models were used to examine the significance of positive RAST's, viral cultures, and heavy passive smoke exposure (cotinine ≥10 ng/ml) in wheezing children relative to controls (Table 2). Children less than age two in this study were 8 times more likely to be wheezing if they had a positive viral culture than if they did not. They were also more likely to be wheezing if they had cotinine levels ≥ 10 ng/ml. Among children over age 2, RAST positivity was strongly associated with wheezing, and viral infection remained a significant risk factor. In addition, the combination of IgE antibody by RAST together with a positive viral culture was present in 14 wheezing children over the age of two as compared to none of the controls, p<0.001.

COTININE · RR = 4.7 (1.0 – 21.3) <2a

(1) TOO LARGE A RANGE TO TELL ANY THING

(1) AS 1.0 IS INCLUDED, NOT SIGN

RR = 0.6 (0.2 – 2.2)

(1) BENEFICIAL EFFECT? HORMESIS?

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DISCUSSION

This study was designed to investigate the prevalence of IgE ab to inhaled allergens, viral infections, and tobacco smoke exposure in infants and children treated for wheezing in a pediatric emergency room. To detect allergen sensitization, serum IgE antibody was measured by RAST because skin testing actively wheezing patients is contraindicated. In our laboratory RAST has been established for five inhalant allergens to which clinic patients react frequently on skin tests (i.e., 97% of skin test positive children with asthma react to one or more of these allergens). In addition, the selection of these allergens is in keeping with the inhaled allergens used in other population surveys (8, 25).

Among wheezing patients under age two, IgE antibody to inhaled allergens was uncommon. This finding is consistent with prospective studies of infants born to allergic parents in whom sensitization to these allergens, determined by skin tests or RAST, was also infrequent prior to age 2 (12, 13). In one of these studies, a cohort of children was followed periodically until age 11 (26). Results showed that exposure to increased levels of dust mite allergen in early childhood was a significant factor in the subsequent development of asthma suggesting that early allergen avoidance may be important for high risk children. Among high risk children are also those who present with atopic dermatitis during infancy who have been shown to have an increased risk for developing respiratory allergies and asthma (27). These children may also present with bronchiolitis. In our study, none of the young wheezing patients under two had signs or symptoms of atopic dermatitis.

An increased prevalence of IgE antibody to inhalant allergens was evident in

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wheezing patients after age two and a significant association between wheezing and sensitization was observed after age four. In keeping with other studies of asthma in children, most of the RAST positive patients were sensitized to dust mite allergen (14, 15), although in drier climates mite sensitization may be less common and IgE antibody to other allergens may be more prevalent. After age 2, a strong correlation between RAST positivity and total IgE levels was evident in the wheezing children. In addition, the increasing prevalence of IgE antibody to inhalant allergens was paralleled by an increase in nasal eosinophilia observed in wheezing patients after age two. Eosinophils are thought to play an important role in the late phase events of immediate hypersensitivity reactions (28). Their presence on nasal smears is also consistent with a response to allergen exposure as judged by their appearance in nasal secretions from grass allergic individuals during the grass pollen season as well as following intranasal allergen provocation (19, 29).

Altogether, viral infections put children both under and over the age of two at significant risk for wheezing. Although the association between viral respiratory infections and wheezing has been described in other epidemiologic investigations (9, 11, 30), this is the first report to compare specific viruses isolated from younger and older children who were treated for wheezing in an emergency room. Our data from wheezing patients under two years support the importance of RSV as a major respiratory pathogen for wheezing in infants (11, 31). None of the virus infected children less than age two however, had IgE antibody to inhaled allergens. In previous studies, an IgE mediated mechanism for wheezing was proposed for some infants with bronchiolitis based on the detection of IgE antibody specific for RSV antigen in nasopharyngeal secretions (32).

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More recently, increased levels of eosinophil cationic protein were also reported in nasal secretions from RSV infected infants (33). In addition, eosinophils have been described in some nasal smears from a study of infants with bronchiolitis; however, a large proportion of these patients had atopic dermatitis (34). By contrast, nasal eosinophilia was not observed in smears from any of the RSV or other virus infected children under age 2 in our study. After the age of 2, virus was isolated from approximately one-third of the washes from children who presented with wheezing, and rhinovirus was the most prevalent pathogen.

The prevalence of tobacco smoke exposure at home was high for wheezing and control patients. Under the age of two, however, a larger proportion of wheezing patients than controls were exposed to tobacco smoke. Studies of smoking trends in the United States indicate that rates of cigarette smoking are higher among lower socioeconomic groups (35). Consistent with this, the income status of our patients, judged by the proportion of the emergency room bill charged to parents, was lower for the wheezing children under age two than for controls. Similarly, the income status of all smoke exposed patients was lower than for non-exposed patients. WHEEZ = f(SOCIAL STATUS) ? AND MOTHERS SMOKE STATUS ?

The most striking finding regarding passive smoke exposure was the large proportion of children under age two who had elevated cotinine levels suggesting of heavy smoke exposure. Although cotinine has been detected in breast milk (36), none of the patients under age two were being breast fed at the time of their enrollment. Thus, we speculate that infants and young children, who are likely to be more physically dependent on their parents, may have a higher risk for heavy exposure if their parents smoke. However, a threshold level at which passive smoke exposure becomes clinically

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significant in causing or aggravating airway hyper-responsiveness has not yet been defined. In addition, when combined with other risk factors, the effects of smoke inhalation may be additive such that lower levels of exposure may have adverse consequences even for older children. Nevertheless, a dose response relationship has been supported by studies indicating higher rates of asthma and increased usage of asthma medications in children exposed to mothers who smoke at least 1/2 pack of cigarettes per day (10, 37). A highly significant correlation between asthma severity and the number of cigarettes smoked by the mother, but not the father, has also been reported (38). Preliminary analysis of our data suggests that children under age two, who were exposed to mothers smoking 1/2 pack per day or more, were highly likely to have salivary cotinine levels ≥10 ng/ml. This did not appear to be the case after age two. WHY NOT ? Additional studies of mother's smoking habits together with cotinine determinations in their children may help to further clarify this relationship.

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CONCLUSIONS

Results from this study indicate that children who present to an emergency room for wheezing prior to age two can be characterized as having a high rate of viral respiratory tract infection, particularly RSV, and a low rate of sensitization to inhalant allergens. In addition, eosinophils were not apparent on nasal smears from any of these IgE antibody negative patients. After age two, viral infections, mostly rhinovirus, remained an important risk factor for wheezing. However, IgE antibody to inhalant allergens, especially to dust mite, together with elevated total IgE levels and nasal eosinophilia, became an increasingly significant finding. Passive smoke exposure was common among wheezing children at all ages. However, high salivary cotinine levels suggesting heavy smoke exposure were significantly more common among the children under age 2. CUT-OFF POINT VERY LOW (10ng/ml)

Most wheezing patients in this study presented to the emergency room with two or more of the risk factors examined. The management of wheezing in the emergency room, however, often focuses exclusively on treatment with medications, after which many patients return home to unaltered environments. In an effort to reduce morbidity, our data would encourage efforts to reduce tobacco smoke exposure at home, especially for young patients, and to consider sensitization to inhaled allergens and allergen avoidance (e.g. to dust mite) in wheezing children at an early age, and particularly in those who begin or continue to wheeze after age two.

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REFERENCES

1. Evans R, Mullally DI, Wilson RW, *et al.* National trends in the morbidity and mortality of asthma in the US. *Chest.* 1987;91:65-74S
2. Gergen PJ, Weiss KB. Changing patterns of asthma hospitalization among children: 1979 to 1987. *JAMA.* 1990;264:1688-1692
3. Guidelines for the Diagnosis and Management of Asthma. US Department of Health and Human Services. Publication No. 91-3042. August, 1991
4. Friday GA, Fireman P. Morbidity and mortality of asthma. *Pediatr Clin North Am.* 1988;35:1149-62
5. Sears MR, Taylor DR, Print CG, *et al.* Regular inhaled beta-agonist treatment in bronchial asthma. *Lancet.* 1990;336:1391-6
6. Barnes PJ. A new approach to the treatment of asthma. *N Eng J Med.* 1989;321:1517-1572
7. Malveaux FJ. Asthma care for the indigent. *J Allergy Clin Immunol.* 1989;83:1029-1031
8. Pollart SM, Chapman MD, Fiocco GP, Rose G, Platts-Mills TAE. Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. *J Allergy Clin Immunol.* 1989;83:875-82
9. Pattemore PK, Johnston SL, Bardin PG. Viruses as precipitants of asthma symptoms. I. Epidemiology. *Clin Exp Allergy.* 1992;22:325-336

2028388212

Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

10. Martinez FD, Cline M, Burrows B. Increased incidence of asthma in children of smoking mothers. *Pediatrics.* 1992;89:21-26

11. Wohl MEB, Chernick V. Bronchiolitis. *Am Rev Respir Dis.* 1987;118:759-781

12. Van Asperen PP, Kemp AS, Mellis CM. A prospective study of the clinical manifestations of atopic disease in infancy. *Acta Pediatr Scand.* 1984;73:80-85

13. Rowntree S, Cogswell JJ, Platts-Mills TAE, Mitchell EB. Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of allergic disease. *Arch Dis Childhood.* 1985;75:633-637

14. Bullock JD, Frick OL. Mite sensitivity in house dust allergic children. Evaluation by clinical and laboratory methods. *Amer J Dis Child.* 1972;123:222-226

15. Warner JO. Mites and asthma in children. *Br J Dis Chest.* 1978;72:79

16. Young S, Le Souef PN, Geelhoed GC, *et al.* The influence of a family history of asthma and parental smoking on airway responsiveness in early infancy. *N Eng J Med.* 1991;324:1168-1173

17. Weiss ST, Tager IB, Speizer FE, Rosner B. Persistent wheeze. *Am Rev Respir Dis.* 1980;122:697-707

18. Evans D, Levison MJ, Feldman CH, *et al.* The impact of passive smoking on emergency room visits of urban children with asthma. *Am Rev Respir Dis.*

2028388213

Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

27. Buffum WP, Settipane GA. Prognosis of asthma in childhood. *Amer. J. Dis. Child.* 1966;112:214-217

28. Durham SR, Kay AB. Eosinophils, bronchial hyperreactivity and late-phase asthmatic reactions. *Clin Allergy.* 1985;15:411-418

29. Murray AB. Nasal secretion eosinophilia in children with grass pollen hay fever. *Canad Med Ass J.* 1971;104:599-600

30. McIntosh K, Ellis EF, Hoffman LS, *et al.* The association of viral and bacterial respiratory infections with exacerbations of wheezing in young asthmatic children. *J. Pediatr.* 1973;82:578-590

31. Henderson FW, Clyde WA, Collier AM, *et al.* The etiologic and epidemiologic spectrum of bronchiolitis in pediatric practice. *J. Pediatrics.* 1979;95:183-190

32. Welliver RC, Wong DT, Sun M, Middleton E, Vaughan RS, Ogra PL. The development of respiratory syncytial virus-specific IgE and the release of histamine in nasopharyngeal secretions after infection. *N Eng J Med.* 1981;305:841-846

33. Garofalo R, Kimpen JL, Welliver RC, Ogra PL. Eosinophil degranulation in the respiratory tract during naturally acquired respiratory syncytial virus infection. *J Pediatr.* 1992;120:28-32

34. Nagayama Y, Sakurai N, Nakahara T, *et al.* Allergic predisposition among infants with bronchiolitis. *J Asthma.* 1987;24:9-17

2028388214

Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

1987;135:567-572

19. Lim D. Eosinophil leukocytes. In: Mygind N, ed. *Nasal Allergy:* Oxford:2nd ed. Blackwell Scientific Publications; 1979:170-181

20. Langone JJ, Van Vunakis H. Radioimmunoassay of nicotine, cotinine, and -(3-pyridyl) - oxo-N-methylbutyramide. *Meth Enzymol.* 1982;84:628-640

21. Van Vunakis H, Tashkin DP, Rigas B, Simmons M, Gjika HB, Clark VA. Relative sensitivity and specificity of salivary and serum cotinine in identifying tobacco-smoking status of self-reported non-smokers and smokers of tobacco and/or marijuana. *Arch. Environ. Health.* 1989;44:53-58

22. Wall MA, Johnson J, Jacob P, Benowitz NL. Cotinine in the serum, saliva, and urine of nonsmokers, passive smokers, and active smokers. *Am. J. Public Health.* 1988;78:699-701

23. Rosner B. Fundamentals of Biostatistics. 3rd edition, Boston, PWS Kent Publishing Co., 1990

24. Etzel RA. A review of the use of saliva cotinine as a marker of tobacco smoke exposure. *Preventive Med.* 1990;19:190-197

25. Dreborg S. Skin tests used for epidemiological studies. *Allergy.* 1989;suppl.10:52-59

26. Sporik R, Holgate ST, Platts-Mills TAE, Cogswell JJ. Exposure to house-dust mite allergen (*Der p* I) and the development of asthma in childhood. *N. Engl J. Med.* 1990;323:502-507

2028388215

Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

35. Pierce JP, Fiore MC, Novotzy TE, Hatziandreu EJ, Davis RM. Trends in cigarette smoking in the United States. *JAMA*. 1989;261:61-65

36. Labrecque M, Marcoux S, Weber JP, Fabia J, Ferron L. Feeding and urine cotinine values in babies whose mothers smoke. *Pediatrics*. 1989;83:93-97

37. Weitzman M, Gortmaker S, Walker DK, Sobol A. Maternal smoking and childhood asthma. *Pediatrics*. 1990;85:505-511

38. Murray AB, Morrison BJ. The effect of cigarette smoke from the mother on bronchial responsiveness and severity of symptoms in children with asthma. *J. Allergy Clin. Immunol.* 1986;77:575-581

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LEGENDS FOR FIGURES

Figure 1A:

IgE antibody to inhalant allergens in sera from wheezing patients (black bars) and controls (hatched bars). In each serum, titers of IgE antibody were measured by RAST to dust mite, cat, cockroach, rye grass, and ragweed allergens. Sera containing ≥40 RAST units/ml (approximately equivalent to 4ng of allergen specific IgE ab/ml) to one or more of the allergens tested were defined as being RAST positive.

Figure 1B:

Percentage of sera from all RAST positive wheezing patients (ages 22 months to 16 yrs), n=48, with IgE ab specific for allergens in dust mite, cat, cockroach (CR), rye grass, and ragweed (RW) extracts.

Figure 2:

Eosinophils in nasal secretions. The percentage of nasal smears containing ≥10 eosinophils per 100 cells counted is shown for wheezing patients (black bars) and controls (hatched bars). These smears were regarded as positive and indicative of at least moderate eosinophilia as judged by a scale proposed by Lim (19).

Figure 3:

Viral detection in nasal washes. RSV (black bars) was detected by a direct fluorescent antibody assay and all antigen positive washes were also positive in culture using a WI-38 fibroblast cell line. Hatched bars indicate rhinovirus and open bars indicate other viral isolates. The percentages of cultures positive for virus were significantly greater for wheezing patients compared to controls for children under age two ($p<0.05$) and over age two ($p<0.05$).

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Figure 4:

Salivary cotinine levels. Solid circles indicate children who were exposed to one or more smokers at home by questionnaire analysis. Open circles represent patients who were not exposed. One 14 year old control patient actively smoked (indicated by a star). Cotinine levels at or above 10ng/ml (long hatched line) have been reported to reflect heavy passive smoke exposure (24). Values below 1ng/ml (short dotted line) fell below the limits of the assay.

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Table 1. Patient Characteristics

	CHILDREN < 2		CHILDREN > 2	
	Wheezing Patients (n=23)	Controls (n=11)	Wheezing Patients (n=76)	Controls (n=46)
Mean age(yr)	0.9	1.2	7.1	9.6
Age range(yr)	0.2-1.8	0.5-1.8	2.0-15.4	2.3-16.1
Male(%)	57%	33%	73%	65%
Black(%)	74%	44%	55%	31%
Payment requirement:*				
100%	9%	60%	19%	34%
10-75%	14%	0%	26%	10%
0%	77%	40%	55%	56%
Family history for allergy	61%	73%	81%	36%
Total IgE (IU/ml)‡	15 (9-25)	5 (3-11)	198(135-206)	28(18-41)

* Payment requirement categories reflect the proportion of the emergency room bill charged to the patient's family based on annual family income. Shown above are the percentage of familes required to pay 100%, from 10-75%, or 0% of their child's bill.

‡ Total IgE levels are reported as geometric means followed by 95% confidence intervals in parentheses.

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Table 2. Odds Ratios for Wheezing Among Children with a Positive RAST, Virus Culture, or Elevated Cotinine Level

Risk Factors	Odds Ratios‡ Under age 2	Over age 2
RAST	ǂ	4.5 (2.0-10.2)***
VIRUS	8.2 (1.3-51.0)*	3.7 (1.3-10.6)*
COTININE ≥10ng/ml	4.7 (1.0-21.3)*	0.6 (0.2-2.2)
RAST and VIRUS	ǂ	10.8 (1.9-59.0)***

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

‡ Reported odds ratios are univariate analyses followed in parentheses by 95% confidence intervals.

ǂ Not determined because of insufficient positive patients.

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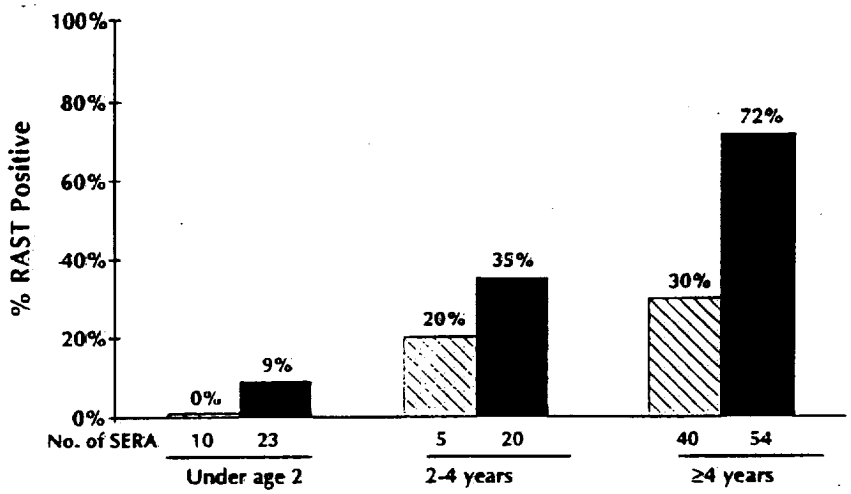

Figure 1A:

IgE antibody to inhalant allergens in sera from wheezing patients (black bars) and controls (hatched bars). In each serum, titers of IgE antibody were measured by RAST to dust mite, cat, cockroach, rye grass, and ragweed allergens. Sera containing ≥40 RAST units/ml (approximately equivalent to 4ng of allergen specific IgE ab/ml) to one or more of the allergens tested were defined as being RAST positive.

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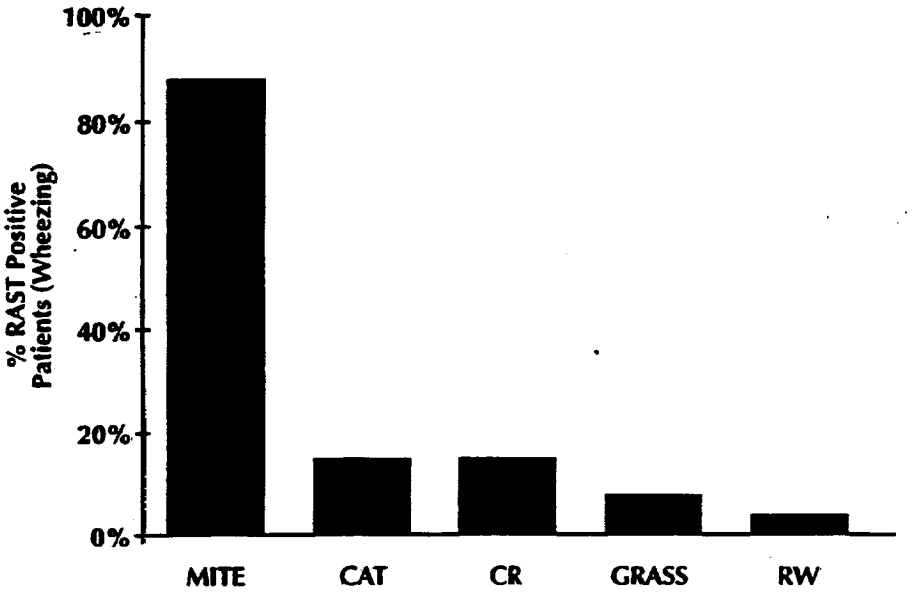

Figure 1B:

Percentage of sera from all RAST positive wheezing patients (ages 22 months to 16 yrs) with IgE ab specific for allergens in dust mite, cat, cockroach (CR), rye grass, and ragweed (RW) extracts.

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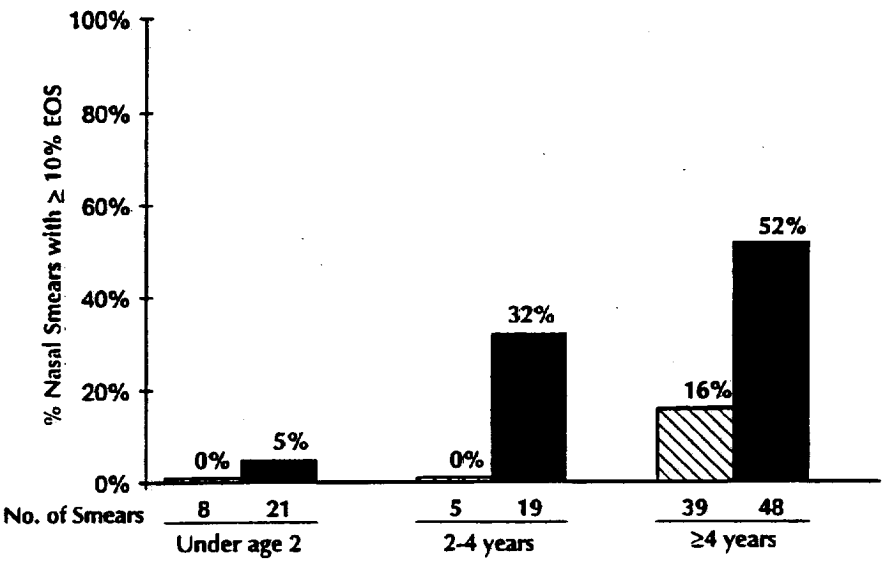

Figure 2:

Eosinophils in nasal secretions. The percentage of nasal smears containing ≥10 eosinophils per 100 cells counted is shown for wheezing patients (black bars) and controls (hatched bars). These smears were regarded as positive and indicative of at least moderate eosinophilia as judged by a scale proposed by Lim (19).

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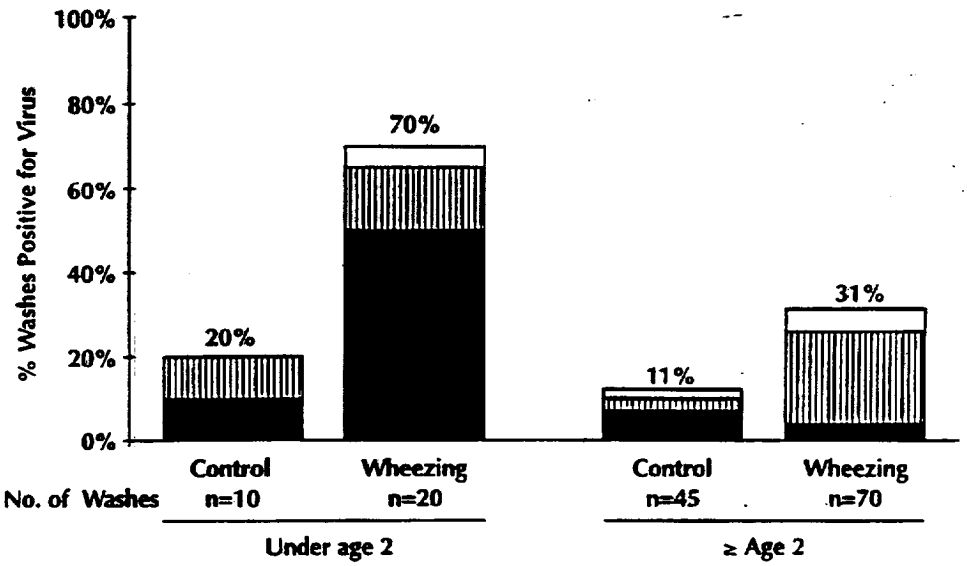

Figure 3:

Viral detection in nasal washes. RSV (black bars) was detected by a direct fluorescent antibody assay and all antigen positive washes were also positive in culture using a WI-38 fibroblast cell line. Hatched bars indicate rhinovirus and open bars indicate other viral isolates. The percentages of cultures positive for virus were significantly greater for wheezing patients compared to controls for children under age two ($p<0.05$) and over age two ($p<0.05$).

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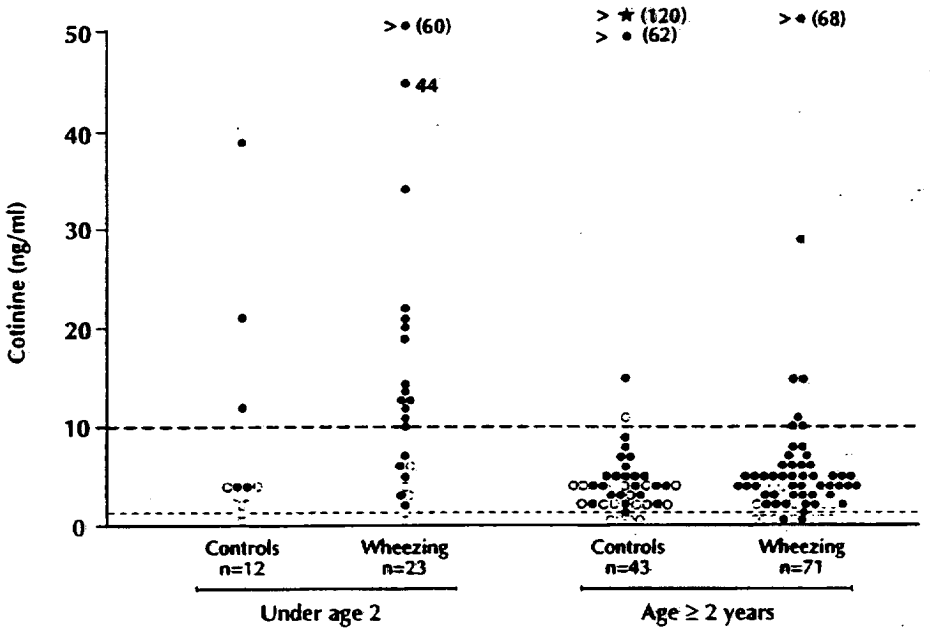

Figure 4:

Salivary cotinine levels. Solid circles indicate children who were exposed to one or more smokers at home by questionnaire analysis. Open circles represent patients who were not exposed. One 14 year old control patient actively smoked (indicated by a star). Cotinine levels at or above 10ng/ml (long hatched line) have been reported to reflect heavy passive smoke exposure (22). Values below 1ng/ml (short dotted line) fell below the limits of the assay.

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DEVELOPMENT OF IMMUNE RESPONSES TO ASPERGILLUS AT AN EARLY AGE IN CHILDREN WITH CYSTIC FIBROSIS

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Running Head: Immune Responses to Aspergillus in C.F.

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ABSTRACT

Although the ability of Aspergillus organisms to colonize the respiratory tract in patients with cystic fibrosis (CF) is well recognized, the contribution of Aspergillus to the disease process is poorly understood. Using sera from 147 CF patients (ages 5-43 years) we measured IgE antibody (ab) to *Aspergillus fumigatus* and 5 common inhalant allergens by radioallergosorbent test (RAST). Total IgE levels and IgG ab to radiolabelled *Asp f* I, an allergen purified from *Aspergillus fumigatus* and a potent inhibitor of protein synthesis, were also measured. Thirty (20%) of the patients had IgE ab to *A. fumigatus* and 22 (15%) of these patients had developed total IgE levels $\geq$ 400 IU/ml so that the diagnosis of Allergic Bronchopulmonary Aspergillosis (ABPA) might be considered. Five of the 22 patients developed these IgE responses by age 5 and fourteen by age 10. The proportion of patients with IgE ab to one or more of the other allergens tested was not significantly different from healthy controls (21% of 147 patients vs. 16% of 32 controls). A striking proportion (84%) of CF sera contained IgG ab to *Asp f* I compared to 6% of sera from control patients and 20% of sera from allergic children with asthma (n=25), only one of whom had IgE ab to *A. fumigatus*. Using additional sera from young CF patients, IgG anti-*Asp f* I ab was detected in 16 of 39 sera (41%) by age 5 increasing to 87 of 89 sera (98%) from patients older than age 10. The early development of immune responses to Aspergillus antigens, including the cytotoxic allergen *Asp f* I, raises the question as to whether Aspergillus, in addition to *Staphylococcus aureus* and *Pseudomonas aeruginosa*, contributes early to progressive lung deterioration in patients with CF.

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KEY WORDS:

Allergic Bronchopulmonary Aspergillosis (ABPA)

Cystic Fibrosis

Allergy

Aspergillus fumigatus

Fungal allergens

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INTRODUCTION

Previous studies of immediate hypersensitivity in patients with cystic fibrosis have consistently demonstrated an increased prevalence of sensitization to Aspergillus allergens (1-4). As judged by skin test responses, sensitization to allergens produced by Aspergillus species, in particular *A. fumigatus*, has been reported to be as high as 31 to 59% in the CF population (2-6). In those patients with IgE anti-Aspergillus ab, the diagnosis of ABPA, an intense inflammatory reaction to Aspergillus in the lungs, is considered when serum total IgE levels exceed 400 IU/ml (or 1000 ng/ml) in conjunction with deterioration in lung function (7). This diagnosis has been reported to affect as many as 10 to 12% of patients with CF (2,4,5) with up to 23% of CF patients meeting most but not all of the criteria (5,6). The increased risk for the development of IgE antibody to Aspergillus in CF patients is presumed to be related to the ability of these fungi to colonize the respiratory tract. Nelson and colleagues grew Aspergillus species from sputum of 57% of patients with CF; however, in other studies, culture yields have been variable and have been reported to be as low as 9 to 11% (2,4,8,9). Serologic responses to Aspergillus antigens generally indicate a higher rate of exposure. Serum precipitins have been reported in 30-51% of patients (2-5,8,9) and IgG anti-Aspergillus ab was demonstrated in 40-70% of CF sera by enzyme linked immunosorbent assay (ELISA) (10,11).

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As yet, it is not clear how early in life exposure and immune responses to Aspergillus antigens develop in CF or whether hypersensitivity to Aspergillus allergens, particularly in patients with high total IgE levels, contributes significantly to deterioration of lung function. In this study, we measured IgE ab to *A. fumigatus* and to 5 common inhalant allergens in sera from patients with CF by RAST and in a portion of these patients by skin prick tests. Results were compared with similar analyses in healthy controls and allergic children with asthma. Total serum IgE levels in all patients and sputum from patients who were skin tested were also obtained. Recently, a major allergen has been purified from *A. fumigatus*, *Asp f* I (12). This allergen shows extensive sequence homology (95%) with a cytotoxin (mitogillin) produced by *Aspergillus restrictus* and both proteins were shown to be potent inhibitors of protein synthesis. Using radiolabelled *Asp f* I, we measured antibody responses to this allergen in serum from all CF patients and controls.

METHODS

Population. Sera from 147 patients (ages 5-43 years, mean = 14.4) were analyzed. Stored sera were available from 97 of these patients (ages 5-26, mean = 13.0) and from an additional 25 younger patients (ages 12 months to 5 years) seen in the cystic fibrosis clinics at the Children's Hospital National Medical Center (Washington, D.C.) and the University of Virginia Children's Medical Center (Charlottesville, VA). Two or more samples, collected at different ages from 30 of these patients, were also available, primarily from children in

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the younger age group. Serum from each patient was obtained during a routine follow-up visit or hospitalization for pulmonary exacerbation and was stored at -20° C. In all patients, the diagnosis of CF had been confirmed by sweat chloride values of > 60 mEq/L by quantitative iontophoresis.

Fifty of the 147 patients (ages 5-43, mean = 18.2) were enrolled consecutively for skin testing together with serum analyses during routine visits to the CF clinic at the University of Virginia. Informed written consent was obtained from patients or their parents. In addition, 25 patients treated at the University of Virginia for asthma (ages 5-18 years, mean = 10.3) in the Pediatric Allergy Clinic and 32 children (ages 5-19 years, mean = 11.1) evaluated in the University of Virginia Pediatric Emergency Room for non-pulmonary diagnoses, and who required venipuncture for management, were enrolled as control patients. The protocol for this study was approved by the Investigational Review Board of the University of Virginia Health Sciences Center.

Immunoassays. Allergen specific IgE antibodies in sera were measured by quantitative RAST using cyanogen bromide activated filter discs coated with either a crude extract of *A. fumigatus* (Greer Laboratories, Lenoir, NC) or one of five other common inhalant allergens. Ten μg of protein from a 100% ammonium sulfate cut of the *A. fumigatus* extract, prepared as previously described (13), was coupled to each disc. The other allergens

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included *Dermatophagoides farinae* mite allergen, cat epithelium, short ragweed pollen, rye grass pollen, and mixed cockroach extracts (Hollister-Stier Laboratory, Spokane, WA). Approximately 0.1-0.5 μg of major allergen (e.g. *Der f* I, *Fel d* I, *Amb a* I, and *Lol p* I from mite, cat, ragweed, and grass extracts, respectively) were coupled to each disc. Allergen coated discs were incubated with dilutions (1/4 and 1/10) of patient's serum and IgE ab bound to allergen was detected using 2 ng of affinity purified ^{125}I-goat anti-human IgE (14). A reference control curve for each assay was established using *D. farinae* coated discs together with serial two-fold dilutions of serum pooled from eight mite allergic patients. This serum pool was allotted an activity of 1000 RAST units/ml by reference to a serum pool established at the National Institute of Biological Standards and Control in London (NIBSC 82/528), which contains 1,800 RAST units of IgE antibody to *D. farinae*. Each RAST unit is approximately equivalent to 0.1 ng of IgE. Dilutions of sera were carried out in 50% horse serum in PBS/TWEEN-20, pH 7.5. Two sera from non-allergic patients and two sera known to contain IgE ab specific for the allergen being tested were run in parallel with each assay. RAST scores of $\geq$ 40 RAST units/ml, or approximately 4 ng of allergen specific IgE ab/ml, were considered positive.

Serum IgG and IgE antibodies to the radiolabelled *Asp f* I were measured in an antigen binding RIA as previously described (12,15). Precipitins to Aspergillus antigens were identified using the standard gel double immunodiffusion template technique using

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unconcentrated serum (16). Each serum was tested against four sources of antigen: *Aspergillus fumigatus* Antigen #1 (20 mg protein/ml), Aspergillin (10 mg protein/ml), and a 1:20 w/v Aspergillus mix (*fumigatus, flavus, glaucus, nidulans,* and *niger*) each from Greer Laboratories, Lenoir, NC, and a 1:20 w/v Aspergillus mix from Hollister-Stier Laboratories, Spokane, WA.

Total serum IgE levels were measured using a mix of two different monoclonal anti-Fc-epsilon antibodies (CIA/E/7/12 and CIA/E/4.15, kindly provided by Dr. Andrew Saxon, UCLA) which were coupled to activated microtiter plates as previously described (14). Bound human IgE was detected using biotin labelled goat anti-IgE antibodies (Kirkegaard and Perry Labs, Gaithersburg, MD). Results were read from a standard curve using serum substandardized against an NIH standard serum containing 900 IU IgE/ml.

Skin testing. Prick skin testing was done on 50 CF patients and 25 allergic asthmatic patients enrolled consecutively in the University of Virginia Cystic Fibrosis and Pediatric Allergy Clinics, respectively. Extracts used were a 1:20 w/v Aspergillus mix, ragweed mix, 9 Southern grass mix, cat epithelium, and American cockroach from Greer Laboratories, Lenoir, NC and a 1:50 w/v *Dermatophagoides farinae* extract from Hollister-Stier Laboratories, Spokane, WA. Histamine (0.25 mg/ml) and saline prick tests were included as positive and negative controls, respectively. All patients had not taken an antihistamine

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for > 72 hours prior to testing. Results were assessed after 15 minutes. A wheal response with a diameter at least 3 mm greater than the negative control was read as positive.

Sputum. When obtained from CF patients, sputum was cultured on Sabouraud's Dextrose Agar with 100 μg/ml of gentamicin and 50 μg/ml of chloramphenicol added to inhibit growth of *Pseudomonas*. Plates were incubated at room temperature and positive fungal cultures were identified in the University of Virginia Hospital microbiology laboratory after 6 weeks. A complete blood count was also obtained from each patient to determine the absolute eosinophil count. Counts > 500 cells per cubic millimeter were considered elevated (7).

Pulmonary function testing. FEV_1 and FVC were performed on an S-model Vitalograph Spirometer (Kansas City, MO). Results were expressed as a percentage of predicted values for age, sex, race, and height based on the standards of Hsu et al (17). Four of the 50 patients enrolled at the University of Virginia were unable to perform spirometry because of acute pulmonary exacerbations. Spirometry values obtained from their most recent clinic visit were used.

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Statistical analysis. Chi square analysis was used to compare the difference between percentages of CF patients and asthma patients with IgE ab to *A. fumigatus* and dust mite allergens. A two sample t-test on the log of geometric means was used to compare the difference between CF patients with and without IgE ab to *A. fumigatus* with respect to titers of IgG ab *Asp f* I.

RESULTS

Sensitization to Aspergillus and common inhalant allergens. As judged by skin prick tests and RAST analyses, allergen specific IgE ab was detected more commonly to Aspergillus allergens in the CF patients than to the other allergens tested (Table I).
Of the 49 patients who had IgE ab to one or more of these six allergens by RAST, 30 (61%) had IgE ab to Aspergillus, 17 of whom had IgE ab to Aspergillus alone.
If one excludes those sensitized to Aspergillus alone, then the prevalence of IgE ab to the inhalant allergens was similar in the CF population and control group (21% and 16%, respectively). By skin test analyses, positive prick tests to Aspergillus were observed in 68% of allergic CF who were skin tested patients compared to 4% of patients with allergic asthma (Fig. 1). By contrast, 80% of the asthmatic subjects had IgE antibody to *D. farinae* dust mite allergen compared to 23% of the skin test positive CF patients. The difference between CF patients and asthma patients with respect to the percentage of individuals with either Aspergillus or dust mite sensitivity was highly significant ($p < 0.001$).

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Total IgE Levels and Suspected ABPA. In sera from the 30 patients who had anti-Aspergillus IgE antibody by RAST, 17 had total IgE levels $\geq$ 400 IU/ml (Table II). Eight of these patients had IgE ab to Aspergillus only. In the absence of IgE ab to Aspergillus, CF patients with inhalant allergy tended to have lower IgE levels. In addition to the 17 patients with total IgE level $\geq$ 400 IU/ml, five other patients with anti-Aspergillus IgE antibody had previous records of total IgE levels > 400 IU/ml which subsequently declined. Three of these five patients had not been treated with steroids. Thus 22 of 30 patients (73%) with IgE ab to Aspergillus, or 15% of the 147 patients ages 5 or older, had or did have immunologic responses characteristic of ABPA. Fourteen of these patients were $\leq$ age 10 and 5 patients were age 5 or younger. The diagnosis of ABPA, however, had been considered clinically by physicians in only 8 of the 22 patients.

Of the 50 patients screened with prick skin tests and total IgE levels, 15 had a positive test for Aspergillus (Table I) and 7 were suspected to have ABPA based on their total IgE levels. Two of the 50 patients had a total blood eosinophil count > 500, but only one of them had a positive Aspergillus skin test and an elevated total IgE (i.e. 690 IU/ml). Measurements of FEV_1 and FVC (and FEV_1/FVC ratios) in this same group did not differentiate the seven patients with suspected ABPA from the other patients.

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IgG antibodies to *A. fumigatus* and sputum cultures. The proportion of CF patients with serum IgG antibodies to the allergen *Asp f* I was 124/147 (84%). All 30 CF patients with serum IgE ab to Aspergillus by RAST had IgG anti-*Asp f* I ab. By contrast, only 5 of 25 (20%) of the allergic patients with asthma and 2 of 32 (6%) of children enrolled as healthy controls had detectable IgG ab to *Asp f* I (Fig 2). Titers of IgG ab to *Asp f* I were significantly higher in patients with IgE ab to *A. fumigatus* by RAST, including patients with total IgE ab $\geq$ 400 IU/ml, (geometric mean = 164.8 units/ml; 95% confidence limits 77.5 to 352.1 units/ml) than in CF patients who did not have IgE ab to Aspergillus (geometric mean = 47.8 units/ml; 95% confidence limits 34.9 to 67.0 units/ml), $p < 0.01$. In 21 sera from the 22 CF patients who had developed immune responses suggesting ABPA, 12 (57%) also showed IgE ab binding to *Asp f* I by antigen binding RIA.

Precipitins were detected in 15 (10%) of the 147 sera tested and twelve of these reacted with antigens present in the *A. fumigatus* Antigen #1 extract. All 15 sera also had IgG ab to *Asp f* I. Eight of the 15 had IgE ab to *A. fumigatus* by RAST and 6 of these had total IgE levels > 400 IU/ml. Forty of the 50 CF patients enrolled for skin testing were able to produce sputum. Aspergillus species were grown in 18 specimens (45%). Only 3 of these patients had total IgE ab $\geq$ 400 IU/ml. *A. fumigatus* was positively identified in 9 of the 18 cultures and came from patients who had high titers (> 100 units) of IgG ab to *Asp f* I.

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Antibody Responses to Aspergillus in young patients. Prior to age 3, the prevalence of IgG ab to the allergen *Asp f* I was low (13%) (Fig. 3). After age 3, a marked increase in the percentage of patients with IgG anti-*Asp f* I ab developed such that after age 10, 98% of patients had detectable antibody responses. A progressive increase with age in titers of IgE ab to Aspergillus by RAST was also evident in sera from 58 CF patients $\leq$ age 10 (Fig. 4A). Fifteen of these patients had developed total IgE levels $\geq$ 400 IU/ml, 14 of whom had IgE ab to Aspergillus (Fig. 4B). In Figure 4A, titers of IgE ab to Aspergillus in serial serum samples (connected circles) available from 9 of these patients are also shown. Six of these patients developed titers > 200 RAST units/ml as well as total IgE levels $\geq$ 400 IU/ml. Five patients developed these levels by age 5 (Fig. 4B).

DISCUSSION

Allergens produced by *A. fumigatus*, and other Aspergillus species, have been shown to be potent stimuli for IgE ab production in patients with CF. Our results demonstrate further that these vigorous IgE responses to Aspergillus in CF can develop at an early age as do immune responses to the *A. fumigatus* allergen and cytotoxic protein, *Asp f* I. As judged by prick skin tests and measurements of serum IgE ab, sensitization to Aspergillus allergens in our patient population was markedly increased compared to other common allergens studied (mite, cat, cockroach, rye grass, and ragweed). Similar to previous reports, we were not able to demonstrate that the percentage of CF patients sensitized to these other allergens

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was significantly increased compared to controls (5,18), whereas the proportion of CF patients sensitized to *A. fumigatus* allergen was significantly increased compared to both asthma and control patients. Twenty-two (15%) of patients older than age 5 in our study had developed IgE ab to Aspergillus in association with high total IgE ab ($\geq$ 400 IU/ml) and IgG ab suggesting the possible diagnosis of ABPA in these individuals. These immune responses were detected in 14 of the 22 patients by the age of 10 and in five patients by the age of 5.

In patients with asthma, the diagnosis of ABPA is considered when IgE ab to Aspergillus and high total IgE ab occur together with other clinical and immunological manifestations (e.g. wheezing, recurrent pulmonary infiltrates, central bronchiectasis, peripheral eosinophilia, and serum precipitins) (7). In patients with cystic fibrosis, however, the diagnosis is more challenging. First, recurrent pneumonic infiltrates and bronchiectasis occur, making it difficult to judge pulmonary manifestations of ABPA using standard x-ray techniques (4). Second, airway hyperreactivity requiring treatment with bronchodilators and sometimes steroids is also common in CF and often is associated with infectious processes (19). Third, over time some CF patients with serologic evidence for ABPA can show variable immune responses to Aspergillus (e.g. loss of precipitins and the fluctuations of serum IgE and IgG ab to Aspergillus) as well as a marked decline in their total IgE levels over time without having been treated with steroids (20). In keeping with

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these diagnostic problems, three of the 22 patients in our study who had developed the antibody characteristics of ABPA had shown a spontaneous decline in their total IgE levels without steroid treatment, and only 8 had been clinically suspected to have the disorder. In addition, peripheral blood eosinophilia was not a marked feature in our patients similar to a previous report of CF patients with suspected ABPA (4).

Although it has been difficult to culture Aspergillus fungi from sputum obtained from CF patients, Aspergillus is thought to colonize and persist in the respiratory tract (3,5). Thus, exposure to Aspergillus in the respiratory tract is the most likely explanation for the high incidence of IgE and IgG ab to Aspergillus allergen. Consistent with a high incidence of exposure, 89% of patients in our study had serum IgG ab to *Asp f* I allergen which was very high compared to previous reports of serum IgG ab responses to Aspergillus in CF patients measured by precipitins or ELISA (2-5,7-10). Furthermore, the detection of IgG ab to *Asp f* I in sera from 39% of our patients by age 5 strongly suggests that exposure to Aspergillus fungi begins at an early age. It is possible that genetic factors could predispose CF patients to respond to Aspergillus. However, the CF gene is localized on chromosome 7 (21,22) whereas allergen specific IgE ab responses to ragweed and rye grass allergens have been reported to be associated with HLA-DR loci located on chromosome 6 (23). A gene locus associated with increased IgE ab responses to inhaled allergens has been reported on chromosome 11 (24). Thus, a linkage disequilibrium between CF and allergen specific immune responses appears to be unlikely based on current information.

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The predilection of *Pseudomonas aeruginosa* for the lungs of patients with cystic fibrosis is well recognized (25). The same might be proposed for Aspergillus fungi considering the high rate of immune responses to *Asp f* I and growth of Aspergillus in 45% of sputum cultures in our study. Recently, *Asp f* I has been shown to be an 18 kd secreted protein which is not detected in significant amounts in Aspergillus spores or mycelial components (15). It is also a potent inhibitor of protein synthesis (26). Other toxic agents in *A. fumigatus* extracts have been reported including metabolites with anti-phagocytic properties (27-29) and a proteinase able to induce epithelial cell detachment from basement membrane (30). Although local factors in the lung may create a suitable growth environment for Aspergillus, the secretion of toxins including *Asp f* I may further enhance the ability of Aspergillus to colonize and persist in the respiratory tract. Although the potential pathologic consequences of Aspergillus, thus far, have focused on CF patients who develop vigorous IgE antibody responses, our data raise the question as to whether exposure to Aspergillus may contribute to chronic and gradual pulmonary deterioration in CF.

In practice, the diagnosis of ABPA is often suspected in CF patients who have a positive skin test and/or serum IgE ab to Aspergillus together with a high total IgE level, particularly when a lung infiltrate is present and the patient's respiratory status fails to improve with appropriate antibiotic and supportive therapy. Oral steroids still represent the basic approach to management in these patients, even though long-term courses of steroids

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have well known complications. As yet there is little information available about the efficacy of oral anti-fungal agents, such as ketoconazole and itraconazole, in treating CF patients with suspected ABPA. In the future the use of antifungals which do not cause unacceptable side effects may help clarify the pathophysiologic significance of Aspergillus in these patients. The use of specific anti-fungal treatment would also permit studies to assess whether Aspergillus, because of its ability to secrete *Asp f I* and other cytotoxic metabolites, may also have pathogenic consequences in the lungs of CF patients who were shown to develop immune responses to Aspergillus at an early age.

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REFERENCES

1. Warren CPW, Tai E, Batten JC, Hutchcroft BJ, Pepys J. Cystic fibrosis-immunological reactions to A. fumigatus and common allergens. Clin Allergy 1975; 1:1-12.

2. Nelson LA, Callerame ML, Schwartz RH. Aspergillosis and atopy in cystic fibrosis. Am Rev Respir Dis 1979; 120:863-73.

3. Zeaske R, Bruns T, Fink JN, Greenberger PA, Colby H, Liotta JL, Roberts M. Immune responses to Aspergillus in cystic fibrosis. J Allergy Clin Immunol 1988; 82:73-7.

4. Laufer P, Fink JN, Bruns T, Unger GFR, Kalbfleisch JH, Greenberger PA, Patterson R. Allergic bronchopulmonary Aspergillosis in cystic fibrosis. J Allergy Clin Immunol 1984; 73:44-8.

5. Feanny S, Forsyth S, Corey M, Levison H, Zimmerman B. Allergic bronchopulmonary Aspergillosis in cystic fibrosis: a secretory immune response to a colonizing organism. Ann Allergy 1988; 60:64-8.

6. Valletta EA, Braggion C, Mastella G. Sensitization to Aspergillus and Allergic Bronchopulmonary Aspergillosis in a cystic fibrosis population. Ped Asthma, Allergy & Immunol 1993; 7:43-9.

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7. Greenberger PA, Patterson R. Diagnosis and management of allergic bronchopulmonary Aspergillosis. Ann Allergy 1986; 56:444-48.

8. Mearns M, Longbotton J, Batten J. Precipitating antibodies to *Aspergillus fumigatus* in cystic fibrosis. Lancet 1967; 1:538-9.

9. Schwartz RH, Johnstone DE, Holschaw DS, Dooley RR. Serum precipitins to *Aspergillus fumigatus* in cystic fibrosis. Am J Dis Child 1970; 120:432-3.

10. Edwards JH, Alfaham V, Fifields R, Philpot C, Clement MJ, Goodchild MC. Sequential serologic responses to Aspergillus fumigatus in patients with cystic fibrosis. Use of antigen 'stretching' to delineate IgG and IgE activity. Clin Exp Immunol 1990; 81:101-8.

11. Forsyth KD, Hohmann AW, Martin AJ, Bradley J. IgG antibodies to *Aspergillus fumigatus* in cystic fibrosis: a laboratory correlate of disease activity. Arch Dis Child 1988; 63:953-57.

12. Arruda LK, Platts-Mills TAE, Fox JW, Chapman MD. *Aspergillus fumigatus* allergen I, a major IgE-binding protein, is a member of the Mitogillin family of cytotoxins. J Exp Med 1990; 172:1529-32.

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13. Harvey C, Longbottom JL: Characterization of a major antigenic component of *Aspergillus fumigatus*. Clin Exp Immunol 1986; 65:206-14.

14. Pollart SM, Chapman MD, Fioico GP, Rose G, Platts-Mills TAE: Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. J Allergy Clin Immunol 1989; 83:875-82.

15. Arruda LK, Platts-Mills TAE, Longbottom JL, El-Dahr JM, Chapman MD. *Aspergillus fumigatus*: identification of 16, 18, and 45 KD antigens recognized by human IgG and IgE antibodies and murine monoclonal antibodies. J Allergy Clin Immunol 1992; 89:1166-76.

16. Kurup VP, Fink JN. Evaluation of methods to detect antibodies against *Aspergillus fumigatus*. Am J Clin Pathol 1978; 69:414-7.

17. Hsu KHK, Jenkins DE, Bartholomew PH, Bourhofer E, Thompson V, Tanakawa N, Hsieh GSJ: Ventilatory functions of normal children and young adults--Mexican-American, white and black. I. Spirometry. J Pediatr 1979; 95:14-23.

18. Silverman M, Hobbs FDR, Gordon IRS, Carswell F. Cystic fibrosis, atopy, and airways lability. Arch Dis Child 1978; 53:873-7.

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

19. Stern RC. The primary care physician and the patient with cystic fibrosis. J Pediatr 1989; 114:33-6.

20. Hutcheson PS, Rejent AJ, Slavin RG. Variability in parameters of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. J Allergy Clin Immunol 1991; 88:390-4.

21. Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, Rozmahel R, Cole JL, Kennedy D, Hidaka N, Zsiga M, Buchwald M, Riordan JR, Tsui LC, Collins FS. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science 1989; 245:1059-65.

22. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou J, Drumm ML, Iannuzzi MC, Collins FS, Tsui L. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 1989; 245:1066-73.

23. Marsh DG, Hsu SH, Hussain R, Meyers DA, Friedhoff MS, Bias WB. Genetics of human immune response to allergens. J Allergy Clin Immunol 1980; 65:322-32.

24. Cookson WOCM, Faux JA, Sharp PA, Hopkin JM. Linkage between immunoglobulin E responses underlying asthma and rhinitis and chromosome 11 q. Lancet 1989; 1:1292-5.

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

25. Davis PB. Cystic fibrosis from bench to bedside. N Engl J Med 1991; 325:575-7.

26. Arruda LK, Mann BJ, Chapman MD. Selective expression of a major allergen and cytotoxin, *Asp f* I, in *Aspergillus fumigatus*: implications for the immunopathogenesis of Aspergillus related diseases. J Immunol 1992; 149, 3354-59.

27. Kurup VP, Resnick A, Scribner GH, Gunasekaran M, Rink JN. Enzyme profile and immunochemical characterization of *Aspergillus fumigatus* antigens. J Allergy Clin Immunol 1986; 78:1166-73.

28. Mullbacher A, Waring P, Eichner RD. Identification of an agent in cultures of *Aspergillus fumigatus* displaying anti-phagocytic and immunomodulating activity in vitro. J Gen Microbiol 1985; 131:1251-58.

29. Yokota K, Shimada H, Kamaguchi A, Sakaguchi O. Studies on the toxins of *Aspergillus fumigatus*. Microbiol Immunol 1977; 21:1-16.

30. Robinson BWS, Venaille TJ, Mendis AHW, McAller R. Allergens as proteases: an Aspergillus fumigatus proteinase directly induces human epithelial cell detachment. J. Allergy Clin Immunol 1990; 86:726-31.

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Table I. Allergen Specific IgE Antibody Responses*

	CYSTIC FIBROSIS		CONTROL PATIENTS
A. Allergens Tested	Skin Test ($n=50$)†	RAST($n=147$)‡	RAST ($n=32$)§
Aspergillus species‖	15 (30%)	30 (20%)	1 (3%)
mite (*D. farinae*)	5 (10%)	14 (10%)	4 (13%)
cat	4 (8%)	20 (14%)	1 (3%)
ragweed	8 (16%)	7 (5%)	1 (3%)
rye grass	5 (10%)	10 (7%)	3 (9%)
cockroach	0	0	0
B. Patients with IgE ab to:			
At least one allergen tested	22 (44%)	49 (33%)	5 (16%)
Inhalant allergens	12 (24%)	31 (21%)	5 (16%)
and *Aspergillus*¶			
Aspergillus only	10 (20)%	17 (12%)	0
Inhalant allergens only**	7 (14%)	19 (13%)	4 (12.5%)

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Footnotes for Table I

* The number of positive tests, followed by the percentage of tests positive in parentheses, are shown for each allergen tested.

† Prick skin tests were carried out on 50 patients with cystic fibrosis (≥ age 5) enrolled consecutively at the University of Virginia's Children's Medical Center. A wheal diameter at least 3 mm > than the negative control was regarded as positive.

‡ Sera for RAST analyses were obtained from 97 patients seen at the Children's Hospital National Medical Center, Washington, D.C., and 50 patients seen at the University of Virginia Children's Medical Center. All patients were ≥ age 5. Values ≥ 40 RAST mites/ml were regarded positive.

§ Sera for control patients were obtained from children (≥ age 5) seen in the University of Virginia Pediatric Emergency Room for a chief complaint which did not involve the respiratory tract.

‖ An extract of mixed *Aspergillus* species (*fumigatus, flavus, glaucus, nidulans,* and *niger*) was used for skin testing. An extract of *A. fumigatus* was used for the RAST analyses.

¶ Patients in this group had IgE ab to one or more of the five inhalant allergens tested and some of these patients also had IgE ab to *Aspergillus.*

** Patients in this group had IgE ab to one or more of the five allergens tested, but did not have IgE ab to *Aspergillus.*

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Table II. Correlation between Serum IgE Antibody to Inhalant Allergens and Total IgE Levels in Cystic Fibrosis.

	Total IgE (IU/ml)*		
	>400	100-400	<100
Patients with IgE ab to:	(n = 19)	(n = 24)	(n = 104)
At least one allergen tested	18 (95%)	13 (54%)	18 (17%)
Inhalant allergens and *A. fumigatus*	17 (89%)	7 (29%)	6(6%)
A. fumigatus only	8 (42%)	5 (21%)	4 (4%)
Inhalant allergens only	1 (5%)	6 (25%)	12 (12%)

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Footnotes for Table II

* Total serum IgE levels were measured using a two-site monoclonal antibody RIA specific for F_c-ϵ (12).

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Legends for Figures:

Figure 1: Distribution of skin test results in allergic patients with cystic fibrosis (solid bars) and asthma (hatched bars). Prick skin tests were positive to one or more allergens tested in 22 of 50 CF patients ($\geq$ age 5) enrolled consecutively at the University of Virginia Health Sciences Center. Results in these 22 patients were compared to skin responses in 25 allergic asthmatic children ($\geq$ age 5) tested in the Pediatric Allergy Clinic at the University of Virginia. Allergen extracts used were Aspergillus, dust mite (*D. farinae*), ragweed, rye grass, cat, and cockroach.

Figure 2: Measurements of IgG antibody to *Asp f* I in sera from 147 patients with CF, 25 patients with asthma and 32 controls. IgG antibodies were measured by radio-immunoprecipitation. Sera with IgE antibodies to *A. fumigatus* by RAST are indicated by solid symbols (i.e. *A f* IgE POS). Solid squares indicate patients who also developed a total IgE level $\geq$ 400 IU/ml. Open circles represent patients who were RAST negative to *A. fumigatus* (i.e. *A f* IgE NEG). Sera with $\leq$ 5 units/ml of IgG ab to *Asp f* I (i.e. less than 2 standard deviations above the mean IgG binding to *Asp f I* in control sera) were regarded as negative. Geometric means (indicated by hatched lines) for CF patients who were *A f* IgE POS and *A f* IgE NEG were 164.8 units/ml and 47.8 units/ml, respectively, and were significantly different, $p < 0.01$.

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Figure 3: Prevalence, with respect to age, of serum IgG antibodies to *Asp f* I in patients with cystic fibrosis. Sera from 147 patients $\geq$ age 5 together with sera from 25 patients < age 5 were analyzed. Two or more samples, obtained at different ages, were available from 30 of the patients. The age ranges indicated along the abscissa span periods of 2 years (e.g. 1-2 is the same as 12 months through 2 years, 11 months).

Figure 4a: Development of IgE antibodies to *A. fumigatus* in patients with cystic fibrosis. Results are shown for all sera tested from children 10 years old or younger. Results of serial serum samples available from 9 patients are shown by connected circles. Closed circles represent RAST measurements $\geq$ 40 units/ml (i.e. above hatched line) and were regarded positive. Numbers along the abscissa followed by an X represent sera tested at each age with measurements < 20 RAST units/ml.

Figure 4b: Development of total IgE levels in patients with cystic fibrosis. Results are shown for all sera tested in Figure 4a with serial samples represented by connected symbols. Sera which were positive by RAST to *A. fumigatus* are shown as closed circles. Those which were RAST negative to *A. fumigatus*, but positive to one or more of the five other allergens tested, are indicated by open squares and those negative to all six allergens are indicated by open triangles. The (*) denotes a decline in total IgE observed following treatment of one of the patients with steroids. Numbers along the abscissa followed by a triangle represent sera tested at each age with < 10 IU/ml of total IgE.

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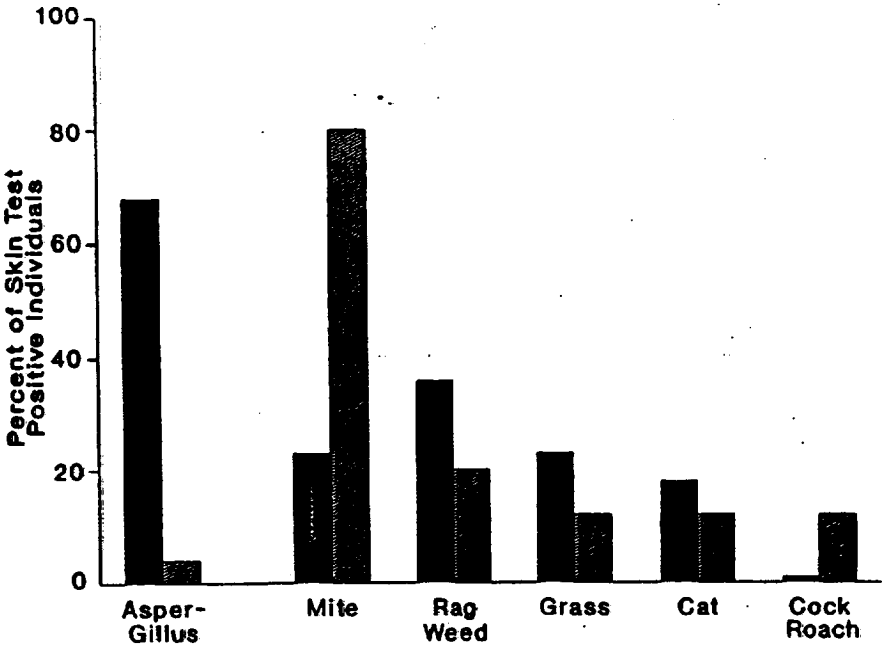

Fig. 1

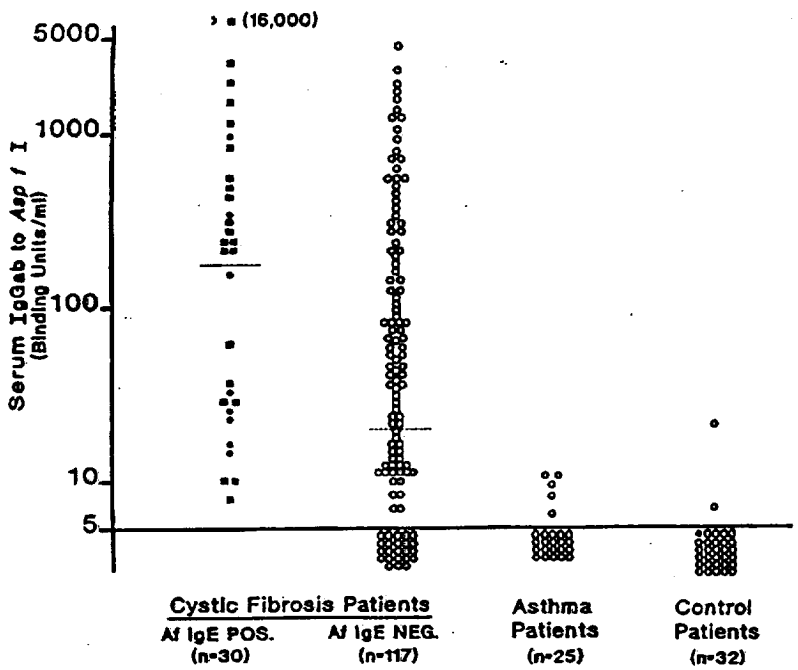

Fig. 2

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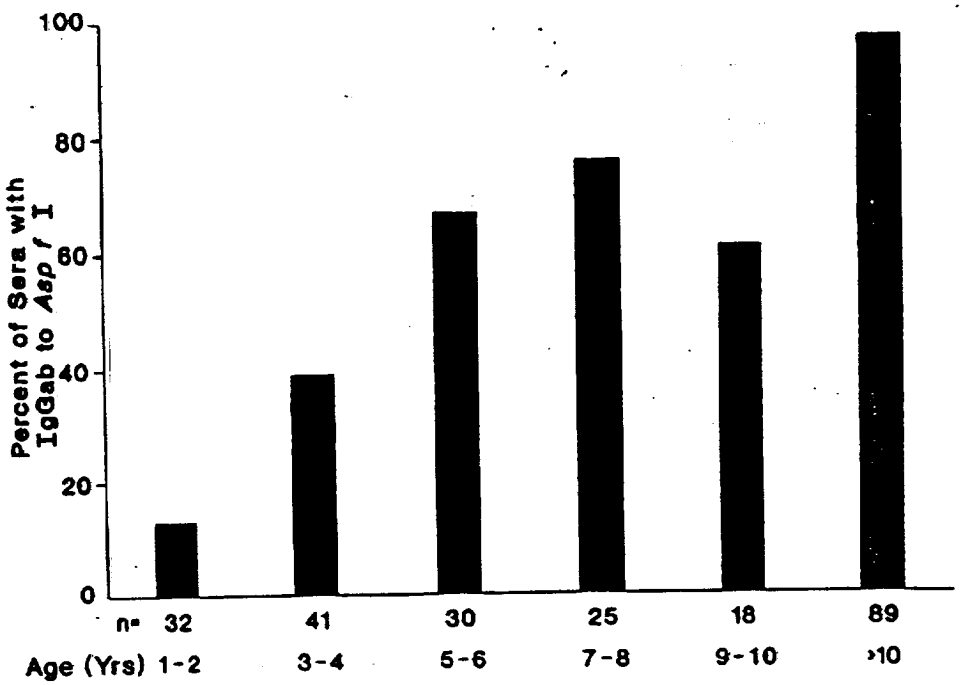

Fig. 3

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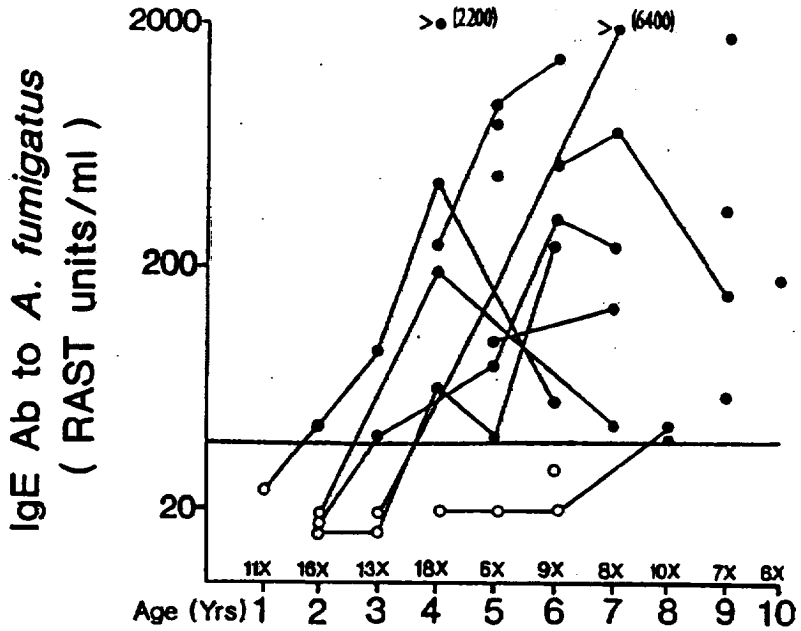

Fig. 4A

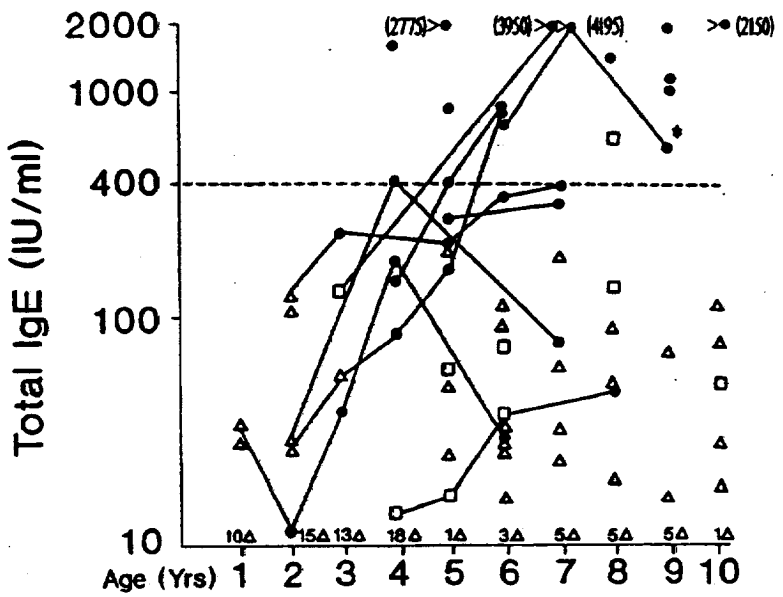

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Fig. 4B

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Early Childhood Inhalant Allergen Sensitization In Atopic Eczema

Running title: Inhalant Allergen Sensitization in AD

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ABSTRACT

Background: The aim of this cross-sectional study was to characterize differences in inhalant allergen sensitization among three age groups of children with atopic dermatitis (AD).

Methods: Sixty-one children (0.5-13 years) with moderate to severe AD were enrolled from an Immunodermatology Clinic and divided into 3 age groups (less than 2, 2-4 years, and greater than 4 years). They were evaluated by history, physical examination, RAST for common inhalant allergens, and total IgE. Thirty-three control patients were enrolled from the outpatient clinics and 57 from a pediatric emergency room.

Results: Inhalant allergen sensitization particularly to dust mite and cat allergens was significantly more prevalent among AD patients than controls ($p<0.001$) as measured by RAST. Under age 2, 38% of AD patients were sensitized to inhalants, and this percentage increased with each successive age group. Significant differences were seen at each age group when AD was compared to controls. Total serum IgE levels were also greatly elevated for each age group. Logistic regression revealed significant odds ratios for the association of AD with log IgE (O.R. 65, 95% CI 12-359), positive RAST (O.R. 13, 95% CI 3.7-49), eosinophil counts (O.R. 15, 95% CI 2.8-85), and family history of allergic disease (O.R. 24, 95% CI 7-86).

Conclusion: The results demonstrate that early aeroallergen sensitization in children with moderate to severe AD is predominantly to the indoor allergens mite and cat. The results suggest that controlled studies on avoidance of inhalant allergens in AD should be started in infancy.

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Keywords: eczema, inhalant allergens, atopic dermatitis, sensitization, childhood, dust mite, cat

Abbreviations: RAST, radioallergosorbent test

GM, geometric mean

CI, confidence interval

AD, atopic dermatitis

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INTRODUCTION

" Despite...evidence of the importance of inhalant allergens in atopic dermatitis, one still sees many patients in whom attention has been directed entirely to removal of offending food or to elimination of psychogenic or infectious factors without benefit in either instance to the patient," wrote Louis Tuft in 1948 in the Journal of Investigative Dermatology (1). Regardless of numerous advances over the last 45 years, the role of inhalant allergens in the pathogenesis of AD is still controversial. The role of food allergens in atopic dermatitis has been more readily accepted with the association demonstrated by extensive work by May, Bock, Sampson and others (2,3). The development of the double blinded placebo controlled food challenge has helped to establish a cause and effect relationship between the ingestion of foods and allergic skin responses (4,5). Challenge testing with inhalants in eczema has been more difficult; however the patch test using inhalant allergen provides a model of the way in which topical exposure could contribute to eczema (6,7). The question arises as to whether aeroallergen enters by inhalation or direct contact with the skin. A role for aeroallergen inhalation has been inferred since 50-80% of children with AD also develop allergic rhinitis and/or asthma (8,9,10). Recent work by Van Reijsen and others has demonstrated that aeroallergens penetrating through the skin can induce a T_H2 cellular response in AD patients (11). Furthermore Langerhans cells in the skin have been shown to express high affinity receptors for IgE in patients with AD who have elevated serum IgE levels(12). These studies strongly suggest that allergen entering through the skin could boost the

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immune response as well as contribute to the skin rash. With increasing emphasis on the role of inhalant allergens in the pathogenesis of AD, few studies have considered the natural history of sensitization to these allergens and most suggest that sensitization occurs after 2-3 years of age (9,13,14). The purpose of the present cross-sectional study was to characterize aeroallergen sensitization in three age groups of children with atopic dermatitis and to contrast these with age-matched controls from two outpatient settings.

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MATERIALS AND METHODS

Sixty-one children with atopic dermatitis were enrolled from the pediatric allergy and immmunodermatology clinics at the University of Virginia. Patients enrolled were from 6 months to thirteen years of age with a mean age of 4.1 years. The diagnosis of atopic dermatitis was made based on the criteria of Hanifin and Rajka following assessment by two physicians (15). Each patient had moderate to severe skin involvement. Evaluation of these patients included allergic history of the patient and their family as well as physical examination. Skin prick testing with *D. farinae* and *D. pteronyssinus* was done and considered positive if the wheal was 3mm greater than the negative control. Serum was assayed for total serum IgE and for IgE antibody by RAST. Thirty-three control patients (control group I) were enrolled from the general pediatric and subspecialty clinics. These patients were from 1 month to 15.9 years old (mean age of 5.4 years), and according to their history had never had eczema or related skin disorders. These patients were evaluated by questionnaire, serum samples for RAST and IgE, and complete blood counts. Fifty-seven control patients (control group II) were enrolled from the pediatric emergency room. They also gave a negative history for atopic dermatitis as well as asthma and were evaluated by questionnaire, serum for RAST, and total IgE analysis. Further studies done with control II patients are reported elsewhere (16).

Allergen-specific IgE to five common inhalant allergens (dust mite, cat, cockroach, rye grass, and ragweed) was measured using cyanogen bromide activated dics coated with

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extracts from Hollister-Stier Laboratories, Inc., Spokane, Washington as previously described (17). Horse serum was used as a diluent to minimize non-specific binding. A positive RAST was defined as >40 RAST units/ml. The units are related to a serum pool obtained from the National Institute of Biological Standards and Control, London, UK (Code #NIBSC 82-528), which was used to establish a *D.farinae* control curve; this unit is equivalent to approximately 0.1ng IgE, thus 40 RAST units is ~4ng/ml of allergen-specific IgE antibody.

Total serum IgE was measured using a two-site monoclonal antibody-based enzyme immunoassay as previously described (monoclonal anti-Fc epsilon antibodies provided by Dr. Andrew Saxon) (18). Complete blood counts to assess total eosinophil counts were measured by peroxidase staining using a cell automated Technicon H1 system in the hospital clinical laboratories. The study was approved by the Human Investigation Committee at the University of Virginia, and all parents of patients gave consent to participate in this study. Statistical analysis was done to compare differences between the control and atopic dermatitis patients using paired T-tests and chi-square test. When samples were small Fishers exact test was used. Logistic regression models were used to assess the differences between AD patients and controls with respect to race, sex, family history, RAST positivity, IgE and eosinophil counts. Regression coefficients from these models were used to estimate odds ratios and 95% confidence intervals. All tests of significance were two tailed (19).

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RESULTS

Demographics

No statistical differences were noted between controls and atopic dermatitis patients with regard to age, race, and sex. These results are summarized in Table 1.

Sensitization to Inhalant Allergen

Overall, 57% of AD patients compared to 9% of control I patients and 22% of control II patients were sensitized to one or more inhalant allergens as measured by RAST ($p<0.001$ for each group). Fifty percent of AD patients were sensitized to dust mite (Figure 1). When comparing AD patients in different age groups, a significant difference was seen in RAST positivity between those children less than 2 and those over 4 ($p<0.01$, Figure 2). In all three age groups, a significant difference was seen in specific IgE antibodies to inhalants between the control groups and the AD group. As judged by RAST results, sensitization to cat allergen was most frequently seen, with 33% of children under 2 being positive (Fig. 3). All children who were positive by RAST to dust mite were also positive by skin test; however, positive skin tests were present in five children under two years of age who had a negative RAST. Thus, by skin testing 41% of the AD children under two were positive to mite. In the older age group (over age 4) 73% of the AD children were sensitized to dust mite and there was an excellent correlation between RAST and skin tests.

In most patients who were positive to dust mite, their RAST specific IgE was very

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elevated, with sixteen having values greater than two thousand (GM=1,919 units). On the average the levels of specific IgE to cat were lower with values in the hundreds of units (GM=305 units) (Figure 4). The levels of IgE ab to cat were very low in the controls with only 2 of 87 having a positive RAST. Among the six children with AD less than 2 years old who were positive to cat, 4 had >200 RAST units/ml.

Total IgE

Measurements of total IgE revealed statistical differences between AD patients and controls among all age groups. Among children under two, the GM of the total IgE for AD patients was 109 IU/ml (95% CI 47 to 253 IU/ml) compared to 3.6 IU/ml (95% CI, 1.4 to 9.4 IU/ml) for control group I and 4.2 IU/ml (95% CI, 1.8 to 9.6 IU/ml) for control group II. Patients with AD from two to four years of age showed GM IgE of 264.5 IU/ml (95% CI 109 to 647 IU/ml); comparable values for the two control groups were 14 IU/ml (95% CI, 5 to 39 IU/ml), and 3.2 IU/ml (95% CI, .8 to 12 IU/ml). This difference was even more pronounced in the patients over four, GM 911 IU/ml (95% CI, 468 to 1758 IU/ml) contrasted with 29 IU/ml (95% CI, 11 to 75 IU/ml) in control group I and 35 IU/ml (95% CI, 23.5 to 51 IU/ml) in control group II. Additionally, absolute eosinophil counts were obtained in a minority of AD patients and for control group I. In 22 AD patients, the mean eosinophil count was 483 /μl compared to 218 /μl in 33 control patients ($p<0.001$).

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Odds Ratios

Several risk factors showed significant univariate odds ratios reflecting their importance in differentiating AD patients from control group I (Table 2). The strongest correlations were seen for log total serum IgE, family history, and positive RAST.

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DISCUSSION

In this study we have demonstrated a high prevalence of IgE antibody to two indoor allergens (dust mite and cat) among children with AD. Furthermore the incidence of inhalant allergen sensitization among children less than two was higher than has been reported previously. Several studies have demonstrated the prevalence of inhalant sensitization among older children (9,13,14,20-22). There have also been case reports suggesting a cause and effect relationship between flares of atopic dermatitis and exposure to inhalant allergens (1,23). Other studies have reported a significant association between IgE ab responses to food allergens and moderate to severe atopic dermatitis (2,3,4,8,9,13). The patients enrolled in the present study were also evaluated for serum IgE antibodies by RAST to milk, soy, peanut, and wheat: in data not presented here, 84% percent of the patients under two were sensitized to one or more foods similar to values reported by others (24,9). Furthermore 38% of the children under 2 years of age had evidence of sensitization to both inhalants and food.

The levels of IgE antibody to dust mite allergen were very high with values of 100-1000 units/ml ~ equivalent to 10-100 ng IgE ab. Even in children less than two, the levels of specific IgE to mite were high in RAST positive patients (range 920-1680 units/ml). Although the levels of IgE antibody to cat allergen were lower in all patients, serum IgE ab to cat was present earlier with six children under two positive. However, judging by skin tests as well as RAST, sensitization to dust mite had also occurred in a large percentage of the very young. The discrepancy between skin test data and RAST

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for dust mite suggests that skin sensitization occurs before a significant rise in serum titers.

Whether the differences between cat and mite sensitization as judged by RAST are related to differences in exposure is not clear. Mite allergen is carried on large particles which are not airborne unless disturbed and settle quickly (25). Cat allergen on the other hand is carried on small particles and is airborne even in undisturbed conditions (26). While some patients may sleep with cats, exposure is more likely to occur via inhalation. Bedding is known to be an important reservoir for mites, but this probably only applies to children over 2 years old since crib bedding is washed regularly and the mattresses are constructed with a plastic covering. It is possible that mite allergen exposure then enters through the skin, is presented via Langerhans cells, and that this route boosts IgE antibody production in the older children. It is also interesting to note the presence of pollen sensitization in some patients under two. Both of the children who had IgE ab to ragweed were born in July just prior to the ragweed season in Virginia. Numerous studies have suggested that the month of birth influences an infants risk of subsequent allergy development (27). However, overall levels of pollen sensitization in the AD patients were low which may reflect the decreasing amount of time spent outdoors or the importance of chronic indoor exposure.

That sensitization to inhalant allergens is common among patients with AD is now fully established. In addition there is sufficient experience from individual cases admitted to hospital or treated with allergen avoidance at home, and from challenge studies to

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suggest that exposure to inhalants is an important factor in the disease. Indeed it is difficult to understand how a patient with >2,000 units of IgE ab to D. pteronyssinus, and excoriated skin lesions would not get symptoms from sleeping in a bed with high levels of dust mite allergen. Beck and Korsgaard demonstrated a dose-response relationship between exposure to house dust mites in bedding and dermatitis in adolescents and adults with AD (28). Our results show that 73% of older children were sensitized and that this process starts under the age of two. At present there is very little controlled evidence to demonstrate the efficacy of avoidance measures for inhalants in the treatment of AD; however this in large part reflects the difficulties of such controlled trials. The present results suggest that the correct design of such a controlled trial would be to enroll very young children presenting with eczema, food sensitivity, and elevated total IgE. The present results imply that avoidance measures should be for both dust mite and cat allergens, and that children should be followed to answer whether intervention can delay sensitization and/or reduce symptoms of eczema.

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REFERENCES

1. Tuft L. Importance of Inhalant Allergens in Atopic Dermatitis. *J Invest Dermatol* 1949; 12:211-219.

2. Bock SA, Lee WY, Remigio LK, May CD. Studies of hypersensitivity reactions to foods in infants and children. *J Allergy Clin Immunol* 1978; 62:327-333.

3. Sampson HA. Role of immediate food hypersensitivity in the pathogenesis of atopic dermatitis. *J Allergy Clin Immunol* 1983; 71:473-480.

4. Sampson HA. Food hypersensitivity and atopic dermatitis: Evaluation of 113 patients. *J Pediatr* 1985; 107:669-675.

5. Bock SA, Sampson HA, Atkins FM, *et al.* Double-blind, placebo-controlled food challenge (DBPCFC) as an office procedure: a manual. *J Allergy Clin Immunol* 1988;82:986-97.

6. Brunijnzeel-Koomen CAFM, van Wichen DF, Spry CJF, *et al.* Active participation of eosinophils in patch test reactions to inhalant allergens in patients with atopic dermatitis. *Br J Dermatol* 1988;118:229-238.

7. Mitchell EB, Crow J, Chapman MD, *et al.* Basophils in allergens-induced patch tests sites in atopic dermatitis. *Lancet* 1982;i:127-130.

8. Adinoff AD, Clark RAF. The allergic nature of atopic dermatitis. *Immunol Allergy Practice* 1989;11:191-202.

9. Guillet G, Guillet MH. Natural history of sensitizations in atopic dermatitis. *Arch Dermatol* 1992;128:187-192.

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10. Salob SP, Atherton DJ. Prevalence of respiratory symptoms in children with atopic dermatitis attending pediatric dermatology clinics. *Pediatr* 1993;91:8-12.

11. Van Reijsen FC, Bruijnzeel-Koomen CAFM, Kalthoff FS, *et al.* Skin-derived aeroallergen-specific T-cell clones of Th2 phenotype in patients with atopic dermatitis. *J Allergy Clin Immunol* 1992;90:184-92.

12. Wang BB, Rieger A, Kilgus O, *et al.* Epidermal langerhans cells from normal human skin bind monomeric IgE via FceRI. *J Experimental Medicine* 1992;175:1353-1365.

13. Rowntree S, Cogswell JJ, Platts-Mills TAE, Mitchell EB. Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of allergic disease. *Arch Dis Child* 1985;60:727-735.

14. Zimmerman B, Chambers C, Forsyth S. Allergy in asthma. *J Allergy Clin Immunol* 1988;81:71-77.

15. Hanifin JM, Lobitz WC. Newer concepts of atopic dermatitis. *Arch Dermatol* 1977;113:663-670.

16. Duff AL, Pomeranz ES, Gelber LE, *et al.* Risk factors for acute wheezing in infants and children: viruses, passive smoke, and IgE antibodies to inhalant allergens. *Pediatr* (in press) 1993.

17. Pollart SM, Chapman MD, Fiocco GP, Rose G, Platts-Mills TAE. Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. *J Allergy Clin Immunol* 1989;83:875-882.

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18. Alshishtawy MM, Abdella AM, Gelber LE, Chapman MD. Asthma in Tanta, Egypt: serologic analysis of total and specific IgE antibody levels and their relationship to parasite infection. *Int Arch Allergy Immunol* 1991;96:348-354.

19. Rosner B. *Fundamentals of Biostatistics*. 3rd edition. Boston: PWS Kent Publishing Co, 1990.

20. Hoffman DR, Yamamoto FY, Geller B, Haddad Z. Specific IgE antibodies in atopic eczema. *J Allergy Clin Immunol* 1975;55:256-267.

21. Ohman S, Johansson SGO. Allergen-specific IgE in atopic dermatitis. *Acta Dermatovener (Stockholm)* 1974;54:283-290.

22. Chapman MD, Rowntree S, Mitchell EB, Di Prisco de Fuenmajor MC, Platts-Mills TAE. Quantitative assessments of IgG and IgE antibodies to inhalant allergens in patients with atopic dermatitis. *J Allergy Clin Immunol* 1983;72:27-33.

23. Clark RAF, Adinoff AD. Aeroallergen contact can exacerbate atopic dermatitis: patch tests as a diagnostic tool. *J Am Acad Dermatol* 1989;21:863-869.

24. Price GW, Duff AL, Farris AH, Platts-Mills TAE, Heymann PW. IgE antibody responses to common food allergens in wheezing infants and children. *J Allergy Clin Immunol* 1993; 91: 173 (abstract).

25. Platts-Mills TAE, Mitchell EB, Rowntree S, Chapman MD, Wilkins SR. The role of dust mite allergens in atopic dermatitis. *Clin Exper Dermatol* 1983;8:233-247.

26. Luczynska CM, Li Y, Chapman MD, Platts-Mills TAE. Airborne concentrations

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and particle size distribution of allergen derived from domestic cats (*Felis domesticus*). Am Rev Respir Dis 1990;141:361-367.

27. Ownby DR. Environmental factors versus genetic determinants of childhood inhalant allergies. *J Allergy Clin Immunol* 1990;86:279-287.

28. Beck HI, Korsgaard JK. Atopic dermatitis and house dust mites. *Br J Dermatol* 1989;120:245-251.

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FIGURE LEGENDS

Figure 1:

The prevalence of IgE antibody by RAST to five common inhalant allergens is shown for patients with atopic dermatitis and controls.

Figure 2:

The effects of age on specific IgE antibody to common inhalants in sera from AD patients (solid bars), control group 1 (right hatched bars), and control group II (vertical striped bars). A positive RAST was >40 U/ml of IgE ab to one or more allergens. Asterisk over AD bars represent significant p values for AD patients versus combined control groups I and II; ** $p<0.01$, and *** $p<0.001$.

Figure 3:

Seven children under two were sensitized to inhalant allergens by RAST. Their ages and IgE ab responses to specific allergens are demonstrated.

Figure 4:

Demonstrates specific RAST units for dust mite and cat allergens in AD patients, control group I, and control group II. AD patients are represented by solid symbols, and controls by open symbols.

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Table I. Patient Characteristics

	AD	CONTROL I	CONTROL II#
number	61	33	57
mean age,years	4.1	5.4	7.9
age range,years	.5-13	.05-15.9	.5-16.1
male/female	62%/38%	45%/55%	59%/41%
race white	41%(42%)*	52%(40%)	67%(44%)
black	53%(53%)	48%(60%)	33%(56%)
other	6%(0%)	0%(0%)	0%(0%)
asthma	42%(28%)	9%(0%)	0%(0%)
rhinitis	28%(17%)	0%(0%)	- -
family history of allergic disease	92%(88%)	33%(20%)	44%(70%)

* Numbers in parenthesis represents values for children under two in each group.

\# Lack of asthma was an inclusion criterion for Control Group II.

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TABLE II. Univariate Odds Ratios For Various Risk Factors Associated With Atopic Dermatitis

RISK FACTORS	Univariate OR	95%CL
female sex	0.50	(.21-1.2)
black race	1.7	(.80-3.7)
family history	24.5	(7.0-85.5)*
positive RAST+	13.5	(3.7-48.9)*
Log IgE	65.7	(12.0-359.0)*
Eosinophil counts#	15.4	(2.8-84.6)*

* Denotes statistical significance.

+ RAST results from common inhalants.

n=22 AD patients and 33 controls

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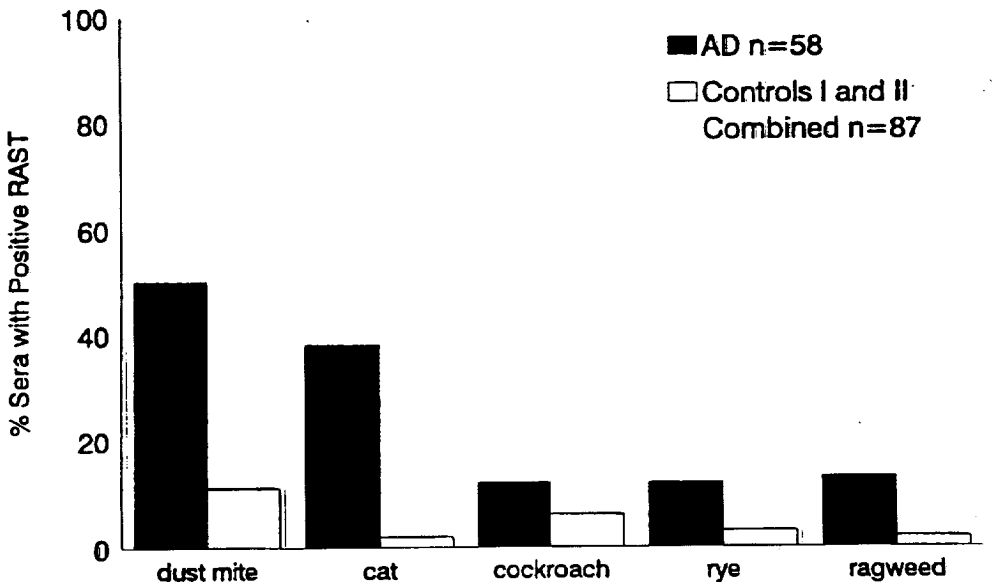

Figure 1:

The prevalence of IgE antibody by RAST to five common inhalant allergens is shown for patients with atopic dermatitis and controls.

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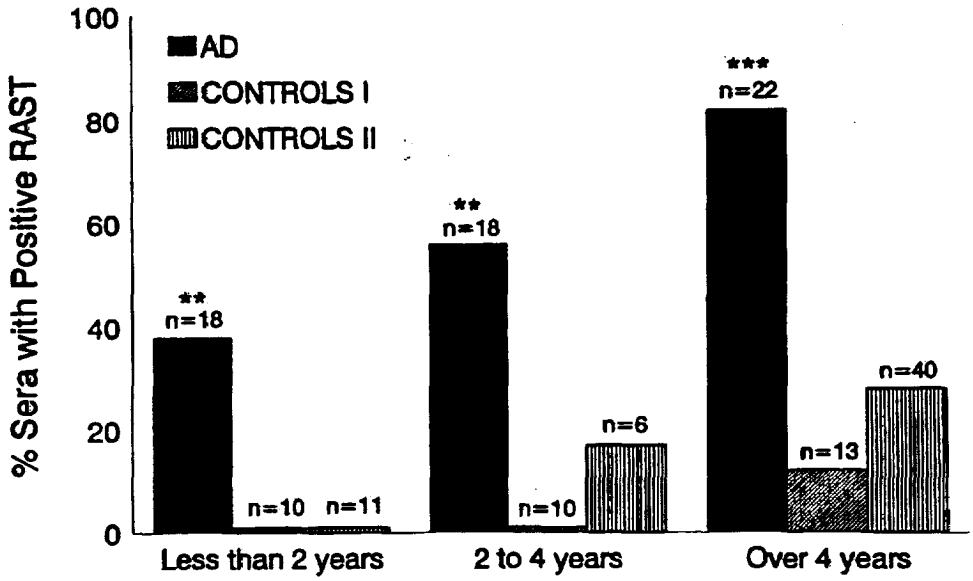

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The effects of age on specific IgE antibody to common inhalants in sera from AD patients (solid bars), control group 1 (right hatched bars), and control group II (vertical striped bars). A positive RAST was >40 U/ml of IgE ab to one or more allergens. Asterisk over AD bars represent significant p values for AD patients versus combined control groups I and II; ** $p<0.01$, and *** $p<0.001$.

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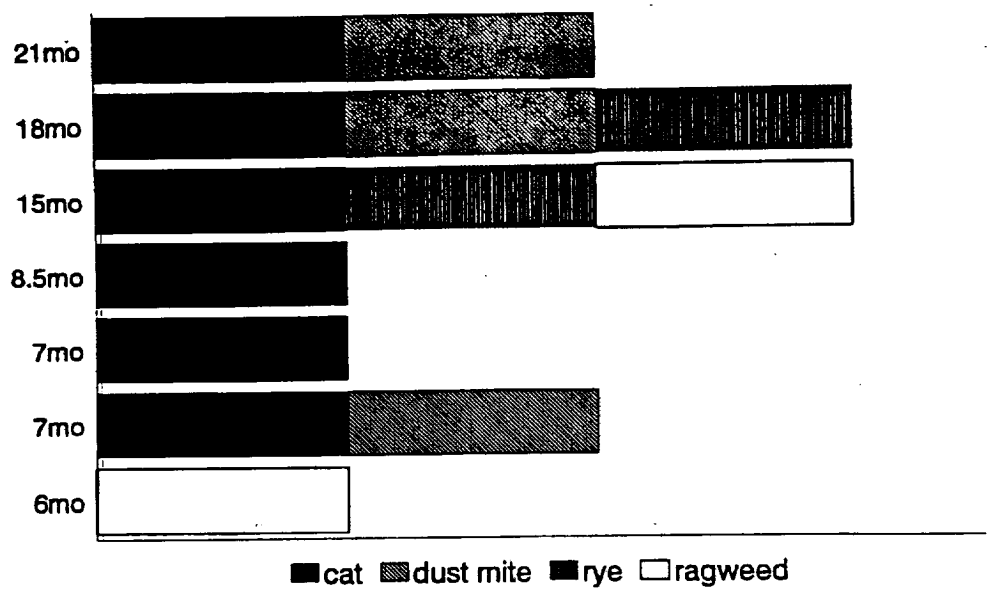

Figure 3:

Seven children under two were sensitized to inhalant allergens by RAST. Their ages and IgE ab responses to specific allergens are demonstrated.

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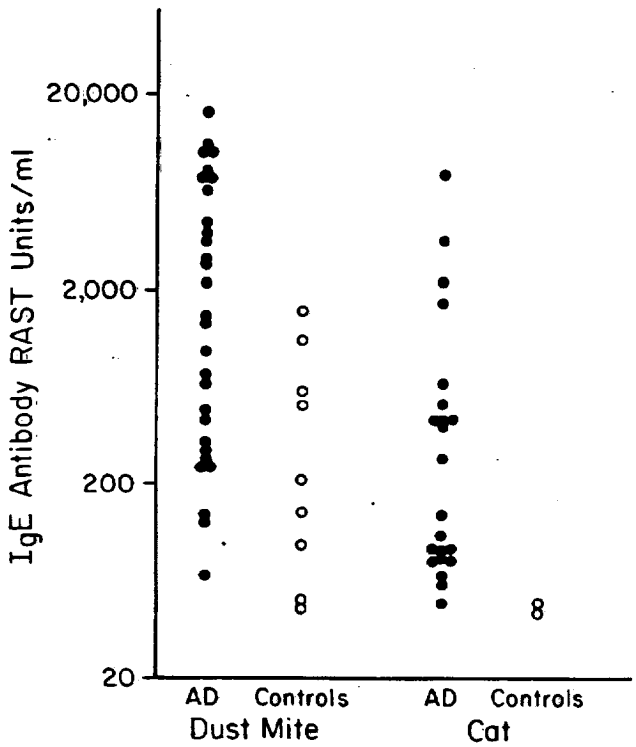

Figure 4:

Demonstrates specific RAST units for dust mite and cat allergens in AD patients, control group I, and control group II. AD patients are represented by solid symbols, and controls by open symbols.

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MONOCLONAL ANTIBODIES TO GROUP II *DERMATOPHAGOIDES spp.* ALLERGENS: MURINE IMMUNE RESPONSE, EPITOPE ANALYSIS AND DEVELOPMENT OF A TWO SITE ELISA

Short title: Monoclonal ab to mite Group II allergens

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Abbreviations used:

Mab	Monoclonal antibody
BSA	Bovine serum albumin
RIA	Radioimmunoassay
ELISA	Enzyme-linked immunosorbent assay
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
HPLC	High-performance liquid chromatography
PBS-T	Phosphate-buffered saline, Ph 7.4, containing 0.05% Tween 20
BBS	Borate-buffered saline, Ph 8.0
SAS	Saturated ammonium sulfate

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ABSTRACT

The Group II allergens are a major cause of sensitization in mite allergic patients. To facilitate the antigenic analysis of Group II allergens and to develop improved methods of allergen detection, we compared IgG anti-Group II antibody responses in inbred mouse strains and raised a panel of monoclonal antibodies (Mab). IgG antibody responses were compared by antigen binding radioimmunoassay and the epitope specificity of the Mab was analyzed by two site binding assays and by cross-inhibition radioimmunoassays. Polyclonal IgG antibodies to Group II were directed against cross-reactive epitopes on *Der p* II and *Der f* II. Comparison of IgG antibody responses in 5 BALB congenic strains, showed that H-2^d mice were poor responders, whereas H-2^b or H-2^k mice made strong IgG anti-Group II responses. The specificity of 9 anti-*Der p* II IgG Mab raised in A/J mice was compared with 7 Mab produced previously. Most Mab (11/16) recognized cross-reactive epitopes on *Der p* II and *Der f* II: three were *Der p* II specific and two showed high binding to *Der f* II. Epitope analysis showed that the Mab defined four cross-reactive, non-overlapping sites on the Group II allergens. Binding of several combinations of mAb was compared and a two site enzyme immunoassay for Group II was developed. Linear regression analysis showed an excellent correlation between results of this assay and Group II radioimmunoassay of house dust samples ($n = 40$, $r = 0.85$, $p < 0.001$). The results show that there are multiple cross-reactive B cell epitopes on Group II allergens. The Group II ELISA has several important applications, including environmental allergen detection, monitoring intervention studies, and standardization of mite allergen extracts.

Key words: Monoclonal antibody, mite allergens, house dust, ELISA, *Dermatophagoides*

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INTRODUCTION

Immunochemical and molecular studies have defined four groups of allergens from *Dermatophagoides pteronyssinus* and *D. farinae*: Group I (25kd, cysteine proteases); Group II (14kd proteins); Group III (~30kd serine proteases); and Group IV (60kd, amylase). The Group I and Group II allergens are the most important cause of sensitization, inducing IgG and IgE antibody responses in 80-95% of mite allergic patients, and each of these allergens (*Der p* I, *Der f* I, *Der p* II and *Der f* II) has been cloned and sequenced (reviewed in 1). There is now strong evidence that sensitization and exposure to mite allergens is an important cause of asthma and an associated cause of atopic dermatitis (1-6). Much of this work has been based on clinical and epidemiologic studies using immunoassays for Group I allergens for environmental allergen detection. These assays have used either *Der p* I or *Der f* I specific monoclonal antibodies (mAb) or monospecific polyclonal antibodies in ELISA or RIA techniques (7-9). Assays for Group I allergens have also been used to assess the quantities and distribution of mite allergens in the air, and to assess the efficacy of physical and chemical mite avoidance procedures (1, 10-18).

Recent studies have compared the environmental distribution and airborne properties of Group II allergens (9, 12, 15, 19). However, measurements of Group II have not been widely adopted because relatively few anti-Group II mAb have been produced and it has only been possible to use the mAb in RIA procedures. The first anti-Group II mAb was raised against *Der p* II and, subsequently, three anti-*Der f* II mAb were produced (20, 21). These mAb recognized common epitopes on *Der p* II and *Der f*

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II and were used to develop affinity purification techniques and solid phase RIA's for Group II. Five other anti-*Der f* II mAb have also recently been produced (23).

The present paper compares the immune response to Group I and Group II allergens in inbred mouse strains and describes the production of a large panel of anti-*Der p* II mAb. Analysis of the epitope specificities of the mAb allowed a two-site ELISA to be developed. The results demonstrate differences between the magnitude and specificity of murine IgG antibody responses to mite allergens and that multiple non-overlapping epitopes on Group II allergens were defined by the mAb. The Group II ELISA showed a very good quantitative correlation with RIA. The results suggest that this assay will be useful in environmental studies, in assessing mite avoidance procedures, and for the standardization of mite allergen extracts.

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MATERIALS AND METHODS

Mite allergens

Group I and Group II allergens were purified from aqueous extract of *D. pteronyssinus* or *D. farinae* culture by mAb affinity chromatography (21). Immunosorbents were prepared using mAb 4C1, for *Der p* I and *Der f* I, and mAb αDpX or mAb 6D6 for *Der p* II and *Der f* II, respectively. Allergens were eluted with 0.005M glycine in 50% ethylene glycol, pH 10.0 (21).

Mouse immunizations

i) Comparison of IgG antibody responses to *Der p* I or *Der p* II.

Groups of 5 animals of six different mouse strains (BALB/c, C57Bl6, C3H, CBA, A/J and AKR) were immunized intraperitoneally, 3 times at 14 day intervals, with 10μg *Der p* I or 10μg *Der p* II in complete Freund's adjuvant (CFA). Mice were bled from the tail vein 14-21 days after the final injection and IgG antibodies in the sera were measured by antigen binding RIA using ^{125}I labelled Group I or Group II allergens.

ii) Comparison of antibody responses to *Der p* II in different BALB strains.

Groups of 3-5 mice (BALB/c, BALB.B, BALB.K, BALB.A10 or BALB.BD.2R) were immunized i.p. 3 times at 14 day intervals with 5μg *Der p* II in CFA, or at 15 day intervals with allergen adsorbed to 250μg aluminium hydroxide gel (Maalox^{R}, Rorer Inc, Fort Washington, PA). IgG antibody responses to *Der p* II or *Der f* II were determined by antigen binding RIA.

Mice were obtained from either Hilltop Laboratories, Scottdale, PA, or from Dr. David Sachs, Harvard University (BALB strains, currently available from Hazelton

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Laboratories, Rockville, MD). The use of animals for this study was approved by the Animal Research Committee of the University of Virginia, under guidelines for the use and care of animals formulated by the National Council for Medical Research.

Monoclonal antibodies to Group II allergens

Six A/J mice were immunized i.p. with 60μg *Der p* II in CFA. At two week intervals mice were boosted twice with 30μg *Der p* II. A final intrasplenic injection of 50μg *Der p* II was given to the mouse demonstrating the highest IgG antibody titer by mAb RIA (1/13,000). Three days later this mouse was sacrificed and its spleen cells were fused with SP_2/O myeloma cells using 37% polyethylene glycol, as described previously (23, 24). Two weeks post-fusion hybrids were screened by ELISA using a 50-80% SAS fraction of *D. pteronyssinus* extract (containing 1300μg/ml *Der p* II). Hybrids producing IgG anti-mite antibodies were tested for binding to ^{125}I *Der p* II and ^{125}I *Der f* II and cloned by limiting dilution. Positive clones were expanded and injected into pristane primed mice for ascites production. Nine anti-*Der p* II mAb were derived from this fusion. Three anti-*Der f* II mAb were also studied (clones 6D6, 7A1 and 4E5) and 4 mAb were kindly provided by either Dr. R. Aalberse (αDpX) or by Dr. H. Okudaira (13A4, 15E11 and 18G8) (20-22).

Antigen-binding RIA

This assay for measuring murine IgG antibody to mite allergens has been described previously (23, 24). Essentially, 100μl diluted mouse antiserum or mAb was incubated for 4hr with 100μl ^{125}I labeled allergen and IgG antibody-antigen complexes were precipitated by the addition of 100μl goat anti-mouse IgG. The precipitates were

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washed three times with BBS, pH 8.0 and counted in a gamma counter.

^{125}I labelling of proteins

Purified mAb or mite allergens were iodinated using a modified chloramine-T technique in which 20μg protein was labeled with 0.5mCi ^{125}I (Amersham Corp., Arlington Heights, IL) (25). The specific activity of ^{125}I *Der p* II or *Der f* II was 5-8μCi/μg protein, whilst that of the mAb or Group I allergens was 30-50μCi/μg.

Epitope analysis of mAb

The epitope specificity of mAb raised against *Der p* II was compared by solid-phase RIA (21, 24). Cyanogen bromide-activated paper discs were coupled with mAb (1μg/disc) and each disc was incubated for 2hr with 0.1ml of a 50-80% SAS fraction of *D. pteronyssinus* or *D. farinae* extract containing 1μg/ml *Der p* II or *Der f* II, respectively. Discs were washed five times with PBS-T and incubated with 500ng cold mAb together with ~5ng ^{125}I labeled mAb for 4hr. After washing, the discs were counted in a gamma counter. All assays were carried out in duplicate, and the sample diluent was 1% BSA PBS-T. Uninhibited binding of ^{125}I mAb to *Der p* II or *Der f* II was calculated from the mean cpm of 4 wells incubated with 1% BSA PBS-T. The percent inhibition of binding of ^{125}I mAb to *Der p* II by cold mAb was calculated as previously (24). Epitope specificity was also analyzed by two site binding RIA using a capture mAb to "present" *Der p* II to a second ^{125}I mAb. Sixteen solid phase mAb were each coupled to CNBr-activated paper discs and incubated for 4hr with 100μl of *D. pteronyssinus* or *D. farinae* extract. After washing, 5ng of each of six ^{125}I labelled mAb (100,000 cpm) was incubated with the discs for 2hr and the discs were washed and counted.

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Biotinylation of mAb

Anti-*Der p* II mAb, 1D8-C4 or 4G7-F1, were purified from ascites by HPLC on a 5ml recombinant protein G sepharose column (Gamma-Bind Plus, Ginex, Gaithersburg, MD) and dialyzed against 0.1M sodium bicarbonate, pH 8.4 overnight at 4°C. For biotinylation, 7.25mg mAb in a volume of 10.35ml (0.7mg/ml), was mixed with 1.24ml of Enzotin (EAB-40 G Enzobiochem, NY) in dimethyl sulfoxide for 4hr at room temperature (7). Enzotin was prepared at the same concentration as the mAb. Unreacted enzotin was removed by dialysis against 4L PBS at 4°C. Biotinylated mAb were titrated using *D. pteronyssinus* (UVA 92/01) and *D. farinae* (UVA 92/02) extracts to determine the optimal dilution for binding in the ELISA (this was 1/15,000). Biotinylated mAb were stored in the dark, at 4°C, diluted 1:15 in 50% glycerol/1% BSA PBS/0.01% thimerosal. Each aliquot contained 42μg/ml biotinylated mAb.

Measurement of Group II allergens by ELISA

The Group II ELISA was modified from the previously published assay for *Der p* I and *Der f* I (7). Plastic microtiter plates were coated overnight with 1μg/well of a 50% SAS cut of mAb 7A1 ascites in 0.05M bicarbonate buffer, pH 9.6 at 4°C. The plates were incubated with 1% BSA PBS-T for 1hr, washed, and incubated with dilutions of allergen or dust extracts. Bound allergen was detected by adding 100μl 1:1000 dilution of biotinylated mAb 1D8 (containing 4.2ng mAb) and, after further washing, 100μl 1:1000 dilution of streptavidin-peroxidase (Sigma, St Louis, MO). The enzyme substrate was 2,2'-azinobis-3-ethyl-benzthiazoline sulfonic acid and the green color reaction was read at 405nm (7).

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The ELISA was quantified using doubling dilutions of *D. pteronyssinus* or *D. farinae* extracts to form control curves from 0.5-250ng/ml. These extracts were prepared by Miles Laboratories, Spokane, WA, from whole mite bodies, and designated UVA 92/01 (*D. pteronyssinus*) and UVA 92/02 (*D. farinae*). Each extract had been substandardized against the Center for Biologics Evaluation and Research mite references, E1-Dp and E1-Df, which were estimated to contain 50μg/ml or 20μg/ml of *Der p* II or *Der f* II, respectively. Stock solutions of the UVA references were prepared at 5μg/ml in 50% glycerol/PBS/1% BSA/0.01% thimerosal and used at a 1:20 dilution to form the first dilution of the control curve (250ng/ml).

To validate the results of the ELISA 40 dust extracts from 20 houses in Sao Paulo, Brazil were assayed by ELISA and the results compared by linear regression analysis with these previously obtained by RIA (19).

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RESULTS

Murine IgG antibody responses to Group I and Group II allergens

Six inbred mouse strains were compared for IgG antibody responses to *Der p* I or *Der p* II (Table I). All responder mouse strains immunized with *Der p* I showed a predominantly species specific response, and in most cases IgG anti-*Der f* I antibody was <20% of the anti-*Der p* I antibody. These results are in keeping with previous data using polyclonal antibodies and mAb from BALB/c mice (23, 24, 26-28). AKR mice failed to make IgG antibodies to *Der p* I, even after prolonged immunization (5 injections of *Der p* I in CFA). In contrast to the response to *Der p* I, IgG antibody responses to *Der p* II were strongly cross-reactive with *Der f* II in all responder strains (Table I). BALB/c mice showed weak or undetectable responses to *Der p* II, which was unexpected, as the original anti-*Der p* II mAb, anti-DpX, had been raised from a BALB.B mouse (20). Antibody responses in congenic BALB strains were compared by immunizing mice with *Der p* II in either CFA or alum (Table II). The results showed that BALB.B, BALB.K, and BALB.A10 mice produced high levels of IgG anti-Group II antibody when immunized in CFA or Alum. BALB/c mice made weak antibody responses following immunization with CFA and made significantly lower titers than the other strains when immunized with *Der p* II in alum. Four BALB/c x BALB.B hybrid mice (BALB.BD.2R) were also immunized with *Der p* II in CFA, using the same immunization protocol, and these mice also had undetectable IgG antibodies to *Der p* II.

Specificity of mAb

A/J mice were used for anti-*Der p* II mAb production, because a BALB/c x A/J

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hybrid (CAF1) can be used for ascites production. Nine mAb were derived from a single fusion using spleen cells from an A/J mouse that had been immunized with *Der p* II in CFA. Binding of these mAb to radiolabeled *Der p* II or *Der f* II was compared with that of 7 other anti-Group II mAb derived from 3 previous fusions using BALB/B, BALB/c or A/J mice (Table III). The RIA results showed that most mAb (11/16) bound to cross-reactive epitopes on both *Der p* II and *Der f* II. Polyclonal mouse IgG anti-*Der p* II antibodies also bound equally well to both allergens. Three clones showed 5-10 fold greater binding to *Der p* II than *Der f* II (3G5-A2, 3G5-H11 and 2B12) and two clones showed significantly higher binding to *Der f* II (15E11 and 4E5). None of the mAb reacted with Group I allergens (data not shown).

Previous studies showed that mAb αDpX, 7A1 and 6D6 defined three non-overlapping cross-reactive epitopes on the Group II allergens (20, 21). The epitope specificity of the 16 mAb used in the present studies was compared by cross-inhibition RIA and by two site binding assay, using cold mab to present allergen to a second ^{125}I labelled mAb. For the cross-inhibition studies, cold mAb was used to inhibit the binding of ^{125}I labelled mAb (1D8 or 4G7) to allergen which was "presented" by a mAb coupled to a paper disc (either 7A1 or αDpX). When allergen was presented by mAb 7A1, the 1D8 and 4G7clones showed 70-98% cross-inhibition, suggesting that they were directed against the same epitope (Fig. 1A). In contrast, several other mAb (e.g. 4E5, 3G5 and 2B12) showed <30% inhibition and appeared to be directed against different epitopes. Similar results were obtained using mAb αDpX on the solid phase, though the levels of inhibition tended to be lower with this mAb (Fig. 1B). Most of the mAb raised in the

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present fusion appeared to have the same specificity as mAb 1D8 or 4G7 and this epitope appeared to be distinct from the three mAb defined sites reported previously (21).

Two site binding assays were carried out to confirm that the epitopes were non-overlapping and to identify mAb combinations that could be used in immunoassays. Six ^{125}I mAb were compared for binding to *Der p* II using each of the 16 mAb on the solid phase. Selected results are shown in Table IV. In each case, there was little or no binding when the solid phase mab was the same as the ^{125}I label. The mAb could be divided into two groups, based on binding of ^{125}I mAb. Some combinations, e.g. using mAb αDpX, 7A1 or 3G5 on the solid phase showed strong binding (20-50,000 cpm bound) using each ^{125}I mAb. Other mAb, e.g. 6D6, 1D8, 4G7 or 13A4, showed binding when used in combination with ^{125}I αDpX, but not with the other five ^{125}I mAb (Table IV). MAb αDpX could be used in a two site assay in combination with any of the other mAb, either on the solid phase or as ^{125}I detecting mAb, with the exception of mAb 15E11 which appeared to have similar specificity to αDpX. The two site binding assays were repeated using *Der f* II and similar patterns of reactivity were obtained (data not shown).

ELISA for Group II allergens

The original mAb RIA for Group II was carried out using anti-*Der f* II mAb 7A1 coupled to CNBr-activated cellulose discs and ^{125}I labeled anti-*Der p* II mAb αDpX for detection (21). Although several combinations of solid-phase mAb coupled to CNBr-activated discs were effective in two site RIA for the detection *Der p* II and *Der f* II, only 3/16 mAb (7A1, αDpX, 13A4) bound to plastic microtiter wells and could be used

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to develop an ELISA (see Table IV). These mAb were used in combination with biotinylated mAb 1D8 or 4G7 and ELISA control curves for either *Der p* II or *Der f* II were compared (Fig. 2A-D). Based on these curves, the combination of mAb 7A1 on the solid phase and biotinylated 1D8, was selected for routine use. This assay gives parallel control curves for both Group II allergens and is ~2 fold more sensitive to *Der f* II than *Der p* II (limits of sensitivity 2-4ng/ml, Fig. 3).

Forty house dust extracts from Sao Paulo, Brazil were analyzed for Group II allergens using both the mAb ELISA and mAb RIA. The results showed very good correlation between Group II levels obtained by ELISA and RIA ($n = 40$, $r = 0.85$, $p < 0.001$) (Fig. 4). The mean within assay coefficient of variation for two separate Group II assays was 12.1% ($n = 69$) and the mean inter assay coefficient of variation on two assays was 16.1% ($n = 15$). In addition, the correlation coefficient between ELISA and RIA for 11 mite extracts from different manufacturers was also very good ($r = 0.95$, $p < 0.001$) (data not shown).

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DISCUSSION

There have been few previous studies on murine immune responses to mite allergens. Studies on IgE antibody responses showed that CBA and C57Bl were high responders to *Der p* I, whereas BALB/c, C3H and AKR were poor responders (30). Most of the studies on IgG antibody responses have been confined to BALB/c mice because this is the preferred strain for mAb production (23, 24, 26-28). The present results show that all the mouse strains tested made high titer, species specific IgG antibody responses to *Der p* I with the exception of AKR mice, which were non-responders. Seven of the eight strains tested, including BALB congenics, also made antibody responses to *Der p* II, which were cross-reactive with *Der f* II. The poor antibody response of BALB/c (H-2^d) mice to Group II allergens has been previously reported and was confirmed by the present studies (21). In addition, BALB.BD.2R mice, which are also H-2^d at the I-A and I-E loci, did not make detectable anti-*Der p* II antibodies. These results are in keeping with a recent study of T cell responses to *Der p* II, in which H-2^d mice made poor T cell responses to *Der p* II, or *Der p* II peptides, whereas H-2^b mice were high responders and H-2^k mice were intermediate (31). In our studies, some H-2^k mice made high levels of IgG antibody to *Der p* II, comparable to those of H-2^b haplotype. Given both the antibody and T cell data, it is difficult to reconcile the production of three high affinity anti-*Der f* II mAb from BALB/c mice (22). The BALB/c derived mAb were produced following prolonged high dose immunization (7 x 30μg *Der f* II in Freund's adjuvant) and, in fact, the antibody and T cell studies each used different immunization protocols, suggesting that the route and dose of

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immunization can significantly affect immune responsiveness to *Der p* II. Analysis of the specificity of anti-Group II mAb produced as part of the present study, together with those produced previously, showed that the mAb define four non-overlapping epitopes, present on both *Der p* II and *Der f* II. These results are consistent with the high degree of amino acid sequence homology between the two allergens (88%, 32).

The development of an ELISA for Group II allergens has several potential applications in asthma and allergy research, including environmental studies, assessment of avoidance procedures and allergen standardization. Immunoassays for the Group I allergens are established as the primary method of assessing mite allergen exposure and threshold values have been proposed as risk factors for the induction of IgE antibody responses and for the exacerbation of asthma attacks (1-6). A good correlation between Group I and Group II allergen levels has been reported in house dust samples from Japan and Brazil, with an ~1:1 ratios of the two groups of allergens in dust extracts (9, 12, 19). The availability of the ELISA will make it possible to compare Group II allergen levels in other parts of the world and to investigate the epidemiologic significance of measuring Group I and Group II allergens. Measurements of both allergens would provide a better measure of "total" allergen load, and the Group II assay has the advantage that it measures allergen from both *D. pteronyssinus* and *D. farinae*.

The Group II ELISA will be particularly useful for mite avoidance studies and for allergen standardization. In terms of avoidance, the assay will be of practical value in assessing the effects of chemical treatments to reduce mite allergen levels, particularly treatments involving allergen denaturation, since it has been established that Group II

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allergens are more resistant to denaturation than Group I (33). Recent studies have shown that measurements of Group II were effective in monitoring the efficacy of benzyl benzoate and tannic acid, currently the two most commonly used acaricidal and chemical treatments (18, 34, 35). Group II measurements should play an important role in the standardization and quality control of mite extracts. The allergen content of mite extract varies significantly with the source material and it is now clear that extracts prepared from whole mite culture usually have a very high Group I;Group II ratio (often >10:1), whereas extracts of pure mite bodies usually have ratios of <2:1 (21). Measurements of either Group of allergens alone may not be representative of the composition or potency of the extract. Preliminary studies of mite body extracts marketed in the U.S., have shown that measurements of both Group I and Group II generally correlate with potency estimates based on skin testing (36). In those studies, it was also clear that some extracts had low major allergen content or had aberrant Group I:Group II ratios. Measurements of both allergens by ELISA provides a simple quantitative method of standardization which will also enable companies to improve their extraction and quality control procedures (37).

Our studies further emphasize the use and applications of mAb in research on mite and other indoor allergens, particularly for assessing environmental exposure. Thus although several other mite allergens have been identified, and in some cases fully sequenced, data on sensitization and exposure to these allergens is limited because few mAb to other allergens are available. The present studies suggest that the panel of mAb will provide useful probes for analyzing B cell epitopes on Group II allergens. The

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ELISA will be a useful adjunct for further epidemiologic studies of mite allergy, for developing improved diagnostic and therapeutic allergen extracts, and for monitoring the efficacy of allergen avoidance procedures, as part of the management of asthma.

Acknowledgements

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References

1. Platts-Mills TAE, Thomas WR, Aalberse RC, Vervloet D, Chapman MD. Dust mite allergens and asthma: Report of a second international worshop. J Allergy Clin Immunol 1992; 89:1046-1060.

2. Lau S, Falkenhorst G, Weber A, Wertmann I, Lind P, Buettner-Goetz P, Wahn U. High mite allergen exposure increases the risk of sensitization in atopic children and young adults. J Allergy Clin Immunol 1989;84:718-725.

3. Sears MR, Hervison GP, Holdaway MD, Hewitt CJ, Flannery EM, Silva PA. The relative risks of sensitivity to grass pollen, house dust mite, and cat dander in the development of childhood asthma. Clin Exp Allergy 1989;19:419-424.

4. Charpin D, Birnbaum J, Haddi E, Genard G, Lanteaume A, Toumi M, Faraj F, Van der Brempt X, Vervloet D. Altitude and allergy to house dust mites: a paradigm of the influence of environmental exposure on allergic sensitization. Am Rev Respir Dis 1991;143:983-986.

5. Sporik RB, Holgate ST, Platts-Mills TAE, Cogswell JJ. Exposure to house dust mite allergen (*Der p* I) and the development of asthma in children. N Engl J Med 1990;323:502-507.

6. Sporik RB, Chapman MD, Platts-Mills TAE. House dust mite exposure as a cause of asthma. Clin Exp Allergy 1992;22:897-906.

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7. Luczynska CM, Arruda LK, Platts-Mills TAE, Miller JD, Lopez M, Chapman MD. A two-site monoclonal antibody ELISA for the quantitation of the major *Dermatophagoides* spp. allergens, *Der p* I and *Der f* I. J Immunol Meth 1989; 118:227-235.

8. Lind P. Enzyme linked immunosorbent assay for major excrement allergens of house dust mite species, *D. pteronyssinus, D. farinae* and *D. microceras*. Allergy 1986;41:442-451.

9. Yasueda H, Mita H, Yui Y, Shida T. Measurement of allergens associated with house dust mite allergy. I. Development of sensitive radioimmunoassays for the two groups of major *Dermatophagoides* mite allergens, *Der* I and *Der* II. Int Arch Allergy Appl Immunol 1990;90:192-190.

10. De Blay F, Heymann PW, Chapman MD, Platts-Mills TAE. Airborne dust mite allergens: Comparison of Group II allergens with Group I mite allergen and cat allergen *Fel d* I. J Allergy Clin Immunol 1991; 88:919-926.

11. Price JA, Pollock J, Little SA, Longbottom JL, Warner JO. Measurements of airborne mite allergen in the homes of asthmatic children. Lancet 1990;336:895-897.

12. Sakaguchi M, Inouye S, Yasueda H, Tatehisa I, Yoshizawa S and Shida T. Measurement of allergens associated with house dust mite allergy. II. Concentration of airborne mite allergens (*Der* I and *Der* II) in the house. Int Arch Allergy Appl Immunol 1990;90:190-193.

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

13. Owen S, Morganstern J, Hepworth J, Woodcock A. Control of house dust mite antigen in bedding. Lancet 1990;335:396-397.

14. Ehnert B, Lau-Shadendorf S, Weber A, Buettner P, Schou C, Wahn U. Reducing domestic exposure to dust mite allergen reduces bronchial hyperreactivity in sensitive children with asthma. J Allergy Clin Immunol 1992;90:135-138.

15. Van Leeuwen J, Aalberse RC. To sieve or not to sieve. *In* "Dust mite allergens and asthma. Report of the Second International Workshop" UCB Institute of Allergy Publications, Brussels, Belgium, pp70-74.

16. McDonald LG, Tovey E. The role of water temperature and laundry procedures in reducing house dust mite populations and allergen content of bedding. J Allergy Clin Immunol 1992;90:599-608.

17. Arshad SH, Matthews S, Gant C, Hide DW. Effect of allergen avoidance on development of allergic disorders in infancy. Lancet 1992;339:1493-1497.

18. Hayden ML, Rose G, Diduch K, Domson P, Chapman MD, Heymann PW, Platts-Mills TAE. Benzyl benzoate moist powder: investigation of acarical activity in cultures and reduction of dust mite allergens in carpets. J Allergy Clin immunol 1992;89:536-545.

19. Arruda K, Rizzo MC, Chapman MD, Fernandez-Caldas E, Baggio D, Platts-Mills TAE, Naspitz CK. Exposure and sensitization to dust mite allergens among asthmatic children in Sao Paulo, Brazil. Clin Exp Allergy 1991; 21:433-439.

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20. Van der Zee JS, van Swieten P, Jansen HM, Aalberse RC. Skin tests and histamine release with P_1-dependent *Dermatophagoides pteronyssinus* body extract and purified P_1. J Allergy Clin Immunol 1988; 81:884-896.

21. Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TAE. Antigenic and structural analysis of Group II allergens (*Der f* II and *Der p* II) from house dust mites (*Dermatophagoides* spp). J Allergy Clin Immunol 1989; 83:1055-1067.

22. Akagawa M, Mori T, Ando T, Okudaira H. Preparation of specific monoclonal antibodies against *Der f* II, one of the major allergens of *Dermatophagoides farinae*. Jpn J Allergol 1991; 40:1239-1242.

23. Chapman MD, Sutherland WM, Platts-Mills TAE. Recognition of two *Dermatophagoides pteronyssinus*-specific epitopes on antigen P_1 by using monoclonal antibodies: binding to each epitope can be inhibited by serum from dust mite-allergic patients. J Immunol 1984; 133:2488-2495.

24. Chapman MD, Heymann PW, Platts-Mills TAE. Epitope mapping of two major inhalant allergens, *Der p* I and *Der f* I, from mites of the genus *Dermatophagoides*. J Immunol 1987; 139:1479-1484.

25. Klinman NR, Taylor RB. General methods for the study of cells and serum during the immune response: the response to dinitrophenol in mice. Clin Exp Immunol 1969; 4:473-487.

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26. Heymann PW, Chapman MD, Platts-Mills TAE. Antigen *Der f* I from the dust mite *Dermatophagoides farinae*: structural comparison with *Der p* I from *Dermatophagoides pteronyssinus* and epitope specificity of murine IgG and human IgE antibodies. J Immunol 1986; 137:2841-2847.

27. Lind P, Hansen OC, Horn N. The binding of mouse hybridoma and human IgE antibodies to the major fecal allergen, *Der p* I, of *Dermatophagoides pteronyssinus*: relative binding site location and species specificity studies by solid-phase inhibition. J Immunol 1988; 140:4256-4262.

28. Le Mao J, Weyer A, Mazie JC, Rouyre S, Marchand F, Le Gall A, David B. Identification of allergenic epitopes on *Der f* I, a major allergen of *Dermatophagoides farinae*, using monoclonal antibodies. Mol Immunol 1992;29:205-211.

29. Vailes LD, Li Y, Bao Y, DeGroot H, Aalberse RC, Chapman MD. Fine specificity of B cell epitopes on *Felis domesticus* allergen I (*Fel d* I): effect of reduction and alkylation or deglycosylation on *Fel d* I structure and antibody binding. Submitted.

30. Stewart GA, Holt PG. Immunogenicity and tolerogenicity of a major house dust mite allergen, *Der p* I from *Dermatophagoides pteronyssinus*, in mice and rats. Int Arch Allergy Appl Immunol 1987;83:44-51.

31. Hoyne GF, Callow MG, Kuo MC, Thomas WR. Characterization of T-cell responses to the house dust mite allergen *Der p* II in mice. Evidence for major and cryptic epitopes. Immunology 1993;78:65-73.

2028388304

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32. Trudinger M, Chua KY, Thomas WR. cDNA encoding the major mite allergen, *Der f* II. Clin Exp Allergy 1991;21:33-40.

33. Lombardero M, Heymann PW, Platts-Mills TAE, Fox JW, Chapman MD. Conformational stability of B cell epitopes on Group I and Group II *Dermatophagoides* spp. allergens. J Immunol 1990; 144:1353-1360.

34. Dietemann A, Bessot JC, Hoyet C, Ott M, Verot A, Pauli G. A double blind, placebo controlled trial of solidified benzyl benzoate applied in dwellings of asthmatic pateints sensitive to mites: clinical efficacy and effect on mite allergens. J Allergy Clin Immunol 1993;91:738-746.

35. Woodfolk JA, Hayden ML, Miller JD, Rose G, Chapman MD, Platts-Mills TAE. Chemical treatment of carpets to reduce allergen: A detailed study of the effects of tannic acid on indoor allergens. Submitted.

36. Chapman MD, Alshistawi M. Standardized mite and cat extracts:analysis of major allergen content by monoclonal immunoassay. J Allergy Clin Immunol 1991;87:179.

37. Platts-Mills TAE, Chapman MD. Allergen Standardization. J Allergy Clin Immunol 1991; 87:621-625.

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LEGENDS TO FIGURES

Figure 1.

Cross-inhibition of the binding of ^{125}I labelled mAb to *Der p* II.

Solid phase mAb 7A1 (Panel A) and αDpX (Panel B) were used to present *Der p* II to 5 - 10ng ^{125}I labelled 4G7 (solid bars) or 1D8 (hatched bars). This binding was inhibited using 500ng cold αDpX (a), 6D6 (b), 7A1 (c), 4E5 (d), 4G7-F1 (e), 4G7-F2 (f), 1D8-C4 (g), 1D8-A6 (h), 3G5-H11 (i), 3G5-A2 (j), 1F9-C6 (k), 2B12-B3 (l), 1E12-E6 (m), 13A4 (n), 15E11 (o) or 18G8 (p). Total radioactivity added (in 0.1ml) = 108,839 cpm (4G7) and 111,840 cpm (1D8).

Figure 2.

ELISA curves for Group II mite allergens using different combinations of mAb.

The curves compare binding of *Der p* II (Panels A and C) or *Der f* II (Panels B and D) using mAb 13A4 (○), 7A1 (●) or αDpX (▲) as capture mAb, and biotinylated mAb 1D8 (Panels A and B) or 4G7 (Panels C and D) for detection.

Figure 3.

ELISA control curves for *Der p* II and *Der f* II using mAb 7A1 on the solid phase and biotinylated mAb 1D8 for detection.

Figure 4.

Correlation between the mAb ELISA and mAb RIA for *Der p* II in 40 house dust extracts collected from 20 houses in Sao Paulo, Brazil.

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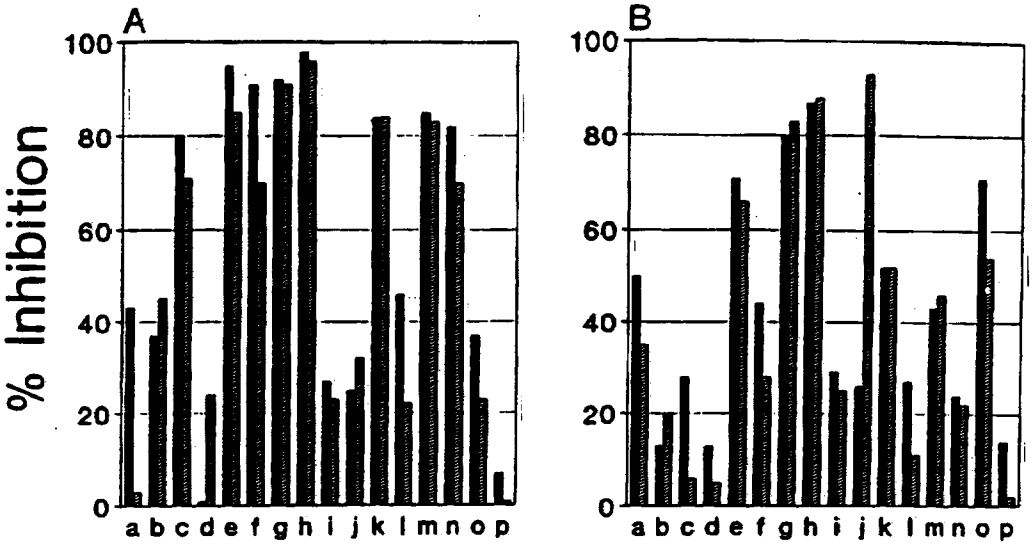

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Cross-inhibition of the binding of ^{125}I labelled mAb to *Der p* II.

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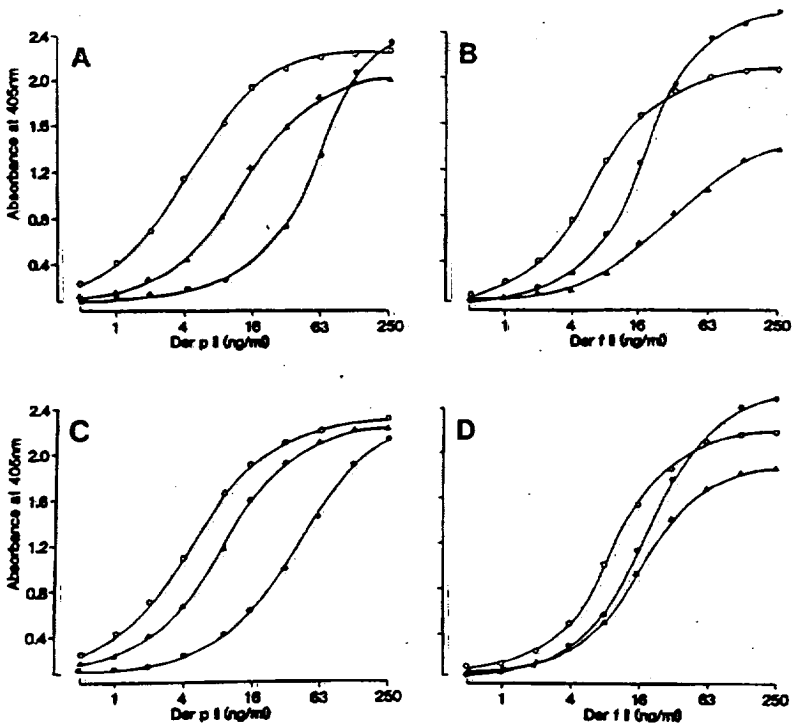

Figure 2.

ELISA curves for Group II mite allergens using different combinations of mAb.

The curves compare binding of *Der p* II (Panels A and C) or *Der f* II (Panels B and D) using mAb 13A4 (○), 7A1 (●) or αDpX (▲) as capture mAb, and biotinylated mAb 1D8 (Panels A and B) or 4G7 (Panels C and D) for detection.

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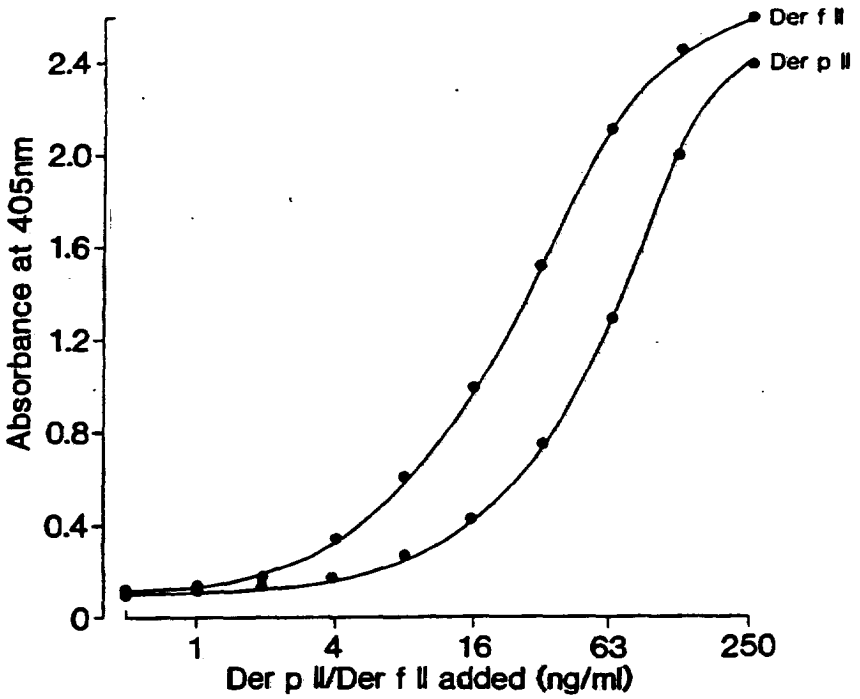

Figure 3.

ELISA control curves for *Der p* II and *Der f* II using mAb 7A1 on the solid phase and biotinylated mAb 1D8 for detection.

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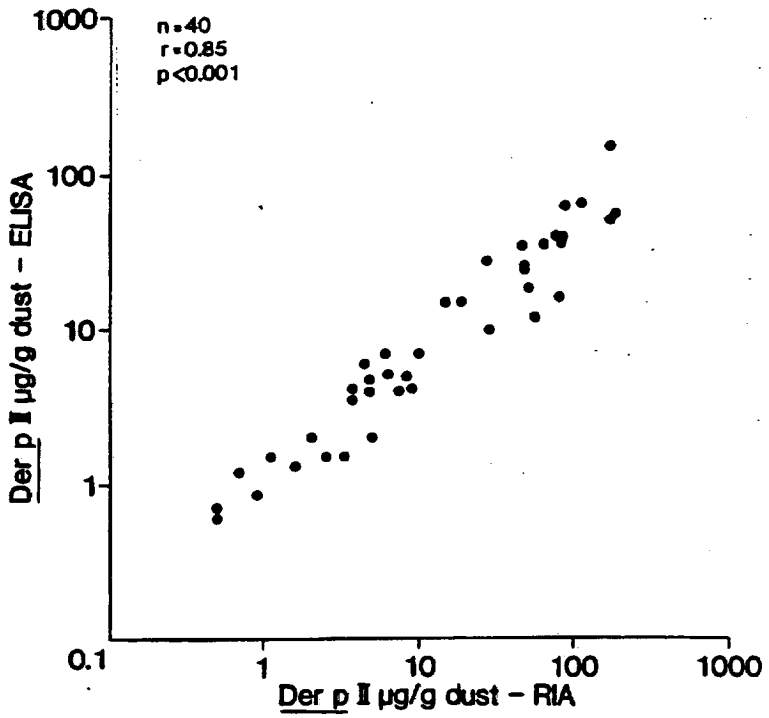

Figure 4.

Correlation between the mAb ELISA and mAb RIA for *Der p* II in 40 house dust extracts collected from 20 houses in Sao Paulo, Brazil.

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TABLE I

Specificity of polyclonal murine IgG ab responses to *Der p* I or *Der p* II*

Strain	H-2 type	μg allergen bound/ml serum+			
		Der p I	*Der f* I	*Der p* II	*Der f* II
BALB/c	d	27.2	1.5	<0.2	<0.2
C57BL6	b	15.0	0.5	11.1	7.7
C3H	k	3.3	0.4	2.9	5.0
CBA	k	10.9	2.1	12.7	16.2
A/J	a	3.5	2.0	3.1	5.0
AKR	k	<0.2	<0.2	n.d.	n.d.

* Groups of 5 mice were immunized 3x with 10μg allergen in CFA.

\+ Determined by antigen binding RIA using ^{125}I labelled allergens. Values represent the geometric mean of assay results on 5 sera.

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TABLE II

Polyclonal IgG anti-Group II ab responses in congenic BALB strains*

Strain	H-2 haplotype	Adjuvant	μg allergen bound/ml serum	
			Der p II	*Der f* II
BALB/c	d	CFA	0.23 ± 0.17	<0.2
		Alum	3.1 ± 0.7	0.5 ± 0.1
BALB.B	b	CFA	12.7 ± 2.4	3.2 ± 0.9
		Alum	18.9 ± 1.7	4.2 ± 0.6
BALB.K	k	CFA	17.7 ± 1.3	9.2 ± 3.5
		Alum	17.4 ± 1.6	4.0 ± 0.3
BALB.A10	a.10	CFA	15.7 ± 1.5	2.7 ± 0.5
		Alum	12.6 ± 1.7	3.2 ± 0.5
BALB.BD(2R)	g	CFA	<0.2	<0.2

* Groups of 3-5 mice were immunized 3x with 5μg *Der p* II in either CFA or alum. Sera were assayed for IgG antibody by antigen binding assay and the results are expressed as the geometric mean ±SEM.

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TABLE III

Monoclonal Antibodies to Dermatophagoides Group II Allergens

mAb [a]	Immunogen	Mouse strain	^{125}I allergen bound (cpm) [#]	
			Der p II	*Der f* II
αDpX [*]	*Der p* II	BALB.B	18,029	11,143
6D6 [§]	*Der f* II	A/J	5,104	12,772
7A1 [§]	*Der f* II	A/J	9,028	11,756
4E5 [§]	*Der f* II	A/J	1,617	22,659
4G7-F1	*Der p* II	A/J	10,738	13,778
4G7-F2	*Der p* II	A/J	12,678	6,336
1D8-C4	*Der p* II	A/J	10,775	16,796
1D8-A6	*Der p* II	A/J	10,423	14,924
3G5-H11	*Der p* II	A/J	10,674	1,105
3G5-A2	*Der p* II	A/J	7,720	808
1F9-C6	*Der p* II	A/J	11,170	5,118
2B12-B3	*Der p* II	A/J	3,024	807
1E12-E6	*Der p* II	A/J	13,946	5,493
15E11 [+]	*Der f* II	BALB/c	3,672	21,422
13A4 [+]	*Der f* II	BALB/c	5,682	17,346
18G8 [+]	*Der f* II	BALB/c	1,336	2,284
Controls:				
6F9 [**]	*Fel d* I	BALB/c	1,344	872
Normal mouse serum		-	1,317	815
Polyclonal mouse anti-serum	*Der p* II	CBA	11,830	15,445

[a] All clones were of the IgG1 isotype, with the exception of 2B12, which was IgG2b.

[#] By antigen binding RIA using mAb ascites at 1/2500 dilution. MAb provided by Dr. R. Aalberse (*), and Dr. H. Okudaira (+), or obtained in a previous study (§, 21).

[**] Control mAb raised against cat allergen *Fel d* I, (29).

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TABLE IV

Comparison of mAb binding to *Der p* II by two site RIA[#]

Solid phase mAb	Immunogen	^{125}I mAb added: αDpX	6D6	4G7	1D8	2B12	1E12
αDpX	*Der p* II	2,109	36,897	40,853 +	36,572 +	22,058	21,108
6D6	*Der f* II	32,320	793	1,662	2,120	1,731	3,121
7A1	*Der f* II	50,728	52,913	45,599 +	41,964 +	30,156	18,603
4E5	*Der f* II	11,869	20,777	23,987	26,100	20,555	9,416
1D8-C4*	*Der p* II	33,494	555	2,271	2,225	2,133	3,043
4G7-F1	*Der p* II	33,256	592	3,315	2,433	2,238	2,488
3G5-H11	*Der p* II	35,047	42,031	30,288	33,408	23,542	15,042
2B12	*Der p* II	24,748	513	1,186	1,490	1,642	2,460
15E11	*Der f* II	1,470	23,995	30,326	22,576	22,716	15,355
13A4	*Der f* II	39,552	48,713	34,774 +	37,512 +	22,663	2,002
PBS control§		444±123	408±69	877±145	855±145	1,154±201	2,268±365

Solid phase mAb were coupled to CNBr-activated paper discs (1 μg/disc). Discs were incubated with *D. pteronyssinus* extract (1μg/ml) overnight, washed and incubated with different ^{125}I second mAb (~110,000 cpm).

* MAb with the same epitope specificity to *Der p* II were grouped and clones with the similar specificity as the 1D8-C4 were as follows: 1D8-A6, 1E12-E6, 1F9-C6, 4G7-F1, 4G7-F2.

§ PBS control results are expressed as the mean cpm ± s.d. for each group of mAb tested.

\+ These combinations of mAb could be used in RIA on plastic microtiter wells and were used to develop an ELISA. The other combinations only showed significant binding in the paper disc assay.

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SENSITIZATION (IgE ANTIBODY) TO FOOD ALLERGENS IN WHEEZING INFANTS AND CHILDREN

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KEYWORDS: asthma, bronchiolitis, food allergy, egg, milk, peanut, soy, atopic dermatitis, IgE

ABBREVIATIONS:

- ab, antibody
- AD, atopic dermatitis
- CI, confidence interval
- DBPC, double-blind placebo-controlled
- GM, geometric mean
- RAST, radioallergosorbent test
- RSV, respiratory syncytial virus

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ABSTRACT

Objective: To assess the prevalence of sensitization to common food allergens in a non-selected population of infants and children treated for wheezing in a pediatric emergency room.

Design: Case control study of actively wheezing children who were compared to children without respiratory tract symptoms as well as to children with atopic dermatitis (AD).

Settings: The Pediatric Emergency Room and the Dermatology Clinic at the University of Virginia.

Patients: Convenience sample of 97 wheezing children (2 months to 16 years), 66 control patients (6 months to 16 years), and 60 children with moderate to severe AD (6 months-13 years).

Measurements and Results: Sensitization (IgE ab) to egg, milk, soy, and peanut allergens were measured in sera from patients by RAST. Positive tests were not common in children seen for wheezing in the emergency room under age 2. The prevalence of positive RASTs increased to 24% in wheezing children ages 2 to 4 and to 31% in children after age 4. After the age of two, both the prevalence as well as titers of serum IgE ab to the food allergens in wheezing patients were similar to controls, but were markedly decreased compared to IgE ab responses in sera from AD patients. However, among children ages 2 to 4, RAST tests for sensitization to food allergens and common inhalant allergens were positive in a similar percentage of wheezing patients (24% and 29%, respectively). In addition, one-third of the patients in this age group had IgE ab responses

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to foods alone. After age 4, however, most wheezing children with positive RASTs (85%) had IgE ab to inhaled allergens.

Conclusion: Children treated for wheezing in the emergency room had a low prevalence of food sensitization and other atopic markers prior to age 2. After age 2, the prevalence of IgE ab to foods increased as did other findings characteristic of atopy and asthma. Between ages 2 to 4, combining tests for IgE ab to common food and inhalant allergens may enhance efforts to identify wheezing patients who are atopic and who are candidates for allergen avoidance measures. After age 4, most wheezing children with IgE ab were sensitized to inhaled allergens and the data for IgE ab to food allergens suggest that evaluations for food hypersensitivity should be more selective in these children.

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INTRODUCTION

Most studies designed to elucidate the relationship between allergy and asthma in children have focused on the role of inhalant allergens. Between 60-85% of school-aged children with asthma are reported to be sensitized to common aeroallergens (1,2) and environmental controls to reduce exposure to these allergens (e.g. to dust mite) can lead to a significant reduction in bronchial hyperreactivity and symptoms (3-5). In contrast, the importance of immediate hypersensitivity to food allergens among children with asthma is not as well defined. Using tests for sensitization combined with DBPC food challenges, several studies have demonstrated that foods can cause infantile and childhood wheezing in selected patients (6-11). However, based on food challenge studies, it does not appear that food allergens are frequently involved in provoking symptoms of asthma (12).

Other studies of food hypersensitivity during childhood have shown that the development of IgE ab to food allergens begins early in life. In prospective studies, up to 30% of children born to allergic parents were found to be sensitized to common food allergens by a year of age (13-15). In the same studies, however, the development of IgE ab to inhaled allergens was delayed and not often detected by skin test or RAST before age two. Among infants who develop IgE ab to foods, the most frequent clinical manifestation of food hypersensitivity is atopic dermatitis (AD). Several reports have shown that these children have an increased risk for developing respiratory allergies, including asthma, later in childhood (16-18). In addition, other studies suggest that a positive skin test for food

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atopic at an early age and have prognostic value as an indicator for the development of allergic respiratory disease (16,19,20). As yet, however, the prevalence of sensitization to common food allergens in infants and children who present with wheezing has never been examined. Previously, in a study of children treated for acute attacks of wheezing in an emergency room, we reported that the prevalence of IgE ab to inhaled allergens was low in those who presented prior to age two (21). After age two, sensitization to these allergens became increasingly common and was significantly increased compared to controls after the age of four. The purpose of the present study was to assess the prevalence of IgE ab to common food allergens by RAST in the same non-selected population of wheezing infants and children.

METHODS

Study Population:

Children in this study included 97 patients who presented to the University of Virginia Pediatric Emergency Room and were given a diagnosis of bronchiolitis or asthma. The age range of these children was from 2 months to 16 years and most (92%) were enrolled during the months of September through June. Wheezing was confirmed by auscultation, and each patient required treatment with at least a beta-2 agonist. Exclusion criteria included a history suggestive of bronchopulmonary dysplasia or recent use of systemic steroids. Sixty-six control patients ranging in age from 6 months to 16 years were also enrolled. These children were enrolled if they had no respiratory tract symptoms and no previous history of wheezing. Sera were also available from 60 children (age 6 months to 13 years) who were seen in the University of Virginia Immunodermatology Clinic for AD.

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These children had moderate to severe AD using criteria set forth by Hanifin and Rajka (22). Their sera were included in this analysis because AD is a common clinical manifestation of food hypersensitivity during childhood.

Historical information was obtained from family members with particular attention to each patient's birth and allergic history as well as the presence of allergic disease in other members of the immediate family (parents and siblings). This study was approved by the human investigation committee at the University of Virginia and informed written consent was obtained from patients or their parents. Additional demographic characteristics of the study groups are presented in Table 1.

Immunoassays:

Sera from enrolled patients were stored at -20° C and subsequently analyzed for IgE ab to egg white, cow's milk, soy and peanut allergens. These allergens were selected after reviewing skin test data from 63 food-sensitized children seen in the pediatric allergy clinic at the University of Virginia. These patients had a positive skin test (i.e. a wheal 3 mm greater than a saline control) to one or more food allergens, and 92% of them could be detected using a screen inclusive of these four allergens. This finding is also consistent with other reports (23).

IgE ab was measured by RAST using cyanogen-bromide activated filter paper discs coated with allergen using methods previously described (24). The starting materials for the preparation of allergen extracts included crude dried egg white (Sigma #A5253), β-lactoglobulin A and B (Sigma #L2506), isolated soy proteins (Ralston-Purina Lot #C6C-E0070) and peanut proteins (defatted Florunner variety). Concentrated extracts of each

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allergen were made by suspension of the crude material in bicarbonate coating buffer (0.1M NaHCO3 + 0.5M NaCl, ph 8.0) followed by an overnight incubation on a rotator. After centrifugation, extracts were dialyzed extensively in coating buffer and then recentrifuged. The protein concentration, determined by Bradford assay, was > 3.0 mg/ml for each extract (25). To optimize the amount of allergen coated to discs, serial four-fold dilutions of each extract were coupled to RAST discs followed by incubation, washing and blocking as previously described (24). Discs were then incubated with pooled sera from at least 5 patients who had strongly positive skin tests for the same allergen being tested. The concentration of each extract showing maximal binding of IgE ab to the discs was chosen for coating discs in this study.

To test sera from patients, discs coated with allergen were incubated with sera diluted 1:4 in duplicate. In order to conserve sera from pediatric patients a modified micro-RAST procedure, utilizing 17 μl of serum per test, was used (26). Bound IgE ab was detected using radiolabelled goat anti-human IgE. Sera from 8-10 patients who were skin test negative served as controls in each assay. Two positive control sera were also included as were two sera with elevated total IgE levels which did not contain specific IgE ab to the allergen tested.

For egg, milk and peanut RAST tests, sera with IgE ab binding $\geq$ 3 standard deviations above the mean counts per minute of 10 negative controls were considered to be positive. For soy, IgE ab binding $\geq$ 4 standard deviations above the mean was read as positive. These criteria were established after comparing RAST and skin test data from 52 food-sensitized clinic patients. The concordance between the RAST assays using these

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food-sensitized clinic patients. The concordance between the RAST assays using these criteria and prick skin tests was 89%, 82%, 86%, and 85% for egg, milk, soy, and peanut, respectively. There were more false negative than false positive RAST responses. The total concordance between these tests (86%) is high and is in agreement with reports from others (27).

Using this food screen, sera with IgE ab to one or more of the allergens tested were considered food RAST positive. To judge titers of specific IgE ab, a control curve was developed for each allergen utilizing serial two-fold dilutions of sera pooled from five patients with positive skin tests to the allergen tested. Because the quantity of allergen coated to discs for egg, milk, soy, and peanut was not likely to be identical, and because the slope of the control curves for these four allergens were also not the same, titers of IgE ab in sera were expressed as a percent of the maximum counts bound at the top of each control curve for each allergen. Measurements of total serum IgE were performed by ELISA as described previously (28). To minimize non-specific IgE binding, sera from patients with high total IgE were diluted to obtain a total IgE level of < 1000 IU/ml.

Statistical Analysis:

To compare differences between the percentage of wheezing, control, and AD patients who were food RAST positive, Chi-square analyses and Fisher's exact test were used. Continuous responses were compared using two sample t-tests. All hypothesis testing was based on two-sided tests of significance (29).

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RESULTS

Among 27 patients who presented with acute wheezing prior to age two, only one had specific IgE ab to one or more food allergens (Fig. 1). This child was 22 months old and was RAST positive to egg. Previously, this patient was also shown to have IgE ab to dust mite (21). None of the control patients in this age group had IgE ab to the food allergens tested. Among the 21 wheezing children from 2 to 4 years of age, sera from 24% were RAST positive compared to 14% of sera from controls. In children over 4 years, the percentage of RAST positive sera did not increase significantly (31%) and was not significantly different from controls (Fig. 1). The increase in specific IgE ab after age 2 was also reflected in the results for individual food allergens (Fig. 2A).

Compared to the findings in wheezing and control patients, a high percentage of sera (84%) from AD patients under age 2 contained IgE ab to one or more food allergens. The prevalence of food-specific IgE antibody in the 2-4 and over 4 age groups with AD was 75% and 63%, respectively. In each age group, the percentage of sera which were food RAST positive was significantly greater in AD patients than in wheezing or control patients ($p < 0.001$ and $p < 0.001$, respectively). This increase was apparent for all four allergens tested (Fig. 2C). Among wheezing and control patients under age 4 as well as AD patients under age 2, sensitization to egg was detected more often than sensitization to milk, soy, or peanut.

The geometric mean (GM) of food-specific IgE ab in each serum was expressed as a percent of the maximal binding of radiolabelled goat anti-human IgE ab at the top of each allergen control curve (Fig. 3). For wheezing patients the GM's of IgE ab to egg, milk, soy, and peanut from those who were RAST positive were 13%, 12%, 20%, and 5%, respectively.

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The GM values for egg, soy, and peanut were similar to controls (13%, 25%, and 9%, respectively). In contrast, the GM of titers to these allergens, except for milk, were significantly lower than the mean titers from RAST positive AD patients (37% for egg, 13% for milk, 44% for soy, and 16% for peanut, $p < 0.001$ for egg, soy, and peanut).

Measurements of IgE ab to inhalant allergens in sera from the wheezing patients were reported in a previous study (21). Shown in Table 2 is an analysis of RAST responses in patients from whom sera were available for measurements of IgE ab to both food and inhalant allergens. Most wheezing children under age two (93%) lacked IgE ab to either food or inhalant allergens. However, between 2 to 4 years of age, 43% of these children were sensitized to food and/or inhalant allergens. In this group, the percentage of patients with IgE ab to food allergens and inhalant allergens was approximately the same (24% and 29%, respectively), and one-third of the RAST positive patients were sensitized to foods only. After age 4, a marked increase in the percentage of patients sensitized to aeroallergens (59%) was observed. This accounted for 85% of the RAST positive patients in this age group. The GM of total serum IgE in children over four who had IgE ab responses only to inhaled allergens was 225 IU/ml. This was significantly higher than the GM of total IgE in sera from children who only had IgE responses to food allergens (GM = 55 IU/ml, $p < 0.001$).

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DISCUSSION

Among children who presented to the emergency room with wheezing prior to age two, the prevalence of serum IgE ab to food allergens in this study was low. Only one patient, who was almost two years old, had a positive RAST test to food allergen (i.e. to egg) and was also previously shown to have IgE ab to dust mite (21). Most of these children presented with their initial attack of wheezing and were diagnosed with bronchiolitis. In addition, viral respiratory pathogens, particularly RSV, were cultured from nasal secretions from 70% of these patients (21). RAST tests were used to measure IgE ab in this study because skin tests are not indicated for actively wheezing patients. Although RAST tests can be less sensitive than skin tests, it is likely that the low prevalence of serum IgE ab to food allergens reflects a low rate of atopy among this group of infants who also had low total IgE levels, a low prevalence of IgE ab to inhaled allergens and only one child (the patient with IgE ab to mite and egg) had nasal eosinophilia. In addition, the concordance between the food RASTs used for this study and skin tests with the corresponding allergens in clinic patients was high (86% overall).

Consistent with other studies of food hypersensitivity in children with eczema, a high prevalence of food sensitization was detected in sera from the children with moderate to severe AD who were under age two (30,31). This finding is pertinent to other reports which, on one hand, suggest that infants with bronchiolitis may have an allergic predisposition or have an increased risk for subsequent wheezing, but which, on the other hand, include a substantial number of children with eczema (32,33). We have also compared our results from the emergency room patients with observations of infants who

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are referred for wheezing to the pediatric allergy clinic. In clinic, patients are almost always referred because they have a history of repeated or persistent episodes of wheezing, an immediate family history for allergy or asthma, and/or eczema. Like infants from the emergency room, these children also have a low prevalence of sensitization to aeroallergens prior to age two; however, up to a third may have positive prick skin test responses to food allergens. Because of their sensitization to food allergens, these children may have a greater risk for developing IgE ab to inhaled allergens and respiratory symptoms later in childhood (16-18). In contrast, it is not clear that children who present with bronchiolitis, but without AD or IgE ab to environmental antigens, have an increased risk for developing asthma as they grow older (34).

Food sensitization increased among wheezing children after age 2 in this study. Many of the children ages 2 to 4 (77%) had a history of prior wheezing and, although four (18%) had a history of eczema, none had active skin disease. In this age group, the RAST tests for foods were positive in approximately the same percentage of wheezing patients as RAST tests for inhalants. Furthermore, 1/3 of the RAST positive children were identified by food tests alone. Thus, combining tests for IgE ab to common food and inhalant allergens in this age group may enhance efforts to identify wheezing patients who are atopic and who, in turn, may benefit from allergen avoidance measures in managing their symptoms.

After age 4, a marked increase in the percentage of patients with IgE ab to inhalant allergens was noted relative to foods. In fact, the majority of patients with serum IgE ab (85%) had positive RAST tests for inhalants. In addition, the prevalence of sensitization and titers of IgE ab to individual food allergens in this group (only 10% of whom had a

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history of AD) did not differ significantly from controls, but were much lower than from AD patients. In other studies, bronchospasm has been reported following DBPC food challenges in selected patients with asthma, more often in those with AD or a history of symptoms consistent with food allergy (6-11). These studies, together with our results, indicate that the evaluation of food hypersensitivity in older children with asthma should be more selective and include those who have a history of AD or food-induced wheezing. In addition, when tests for food sensitization are positive, further evaluation (e.g. food challenge studies) is needed to judge the clinical relevance of a positive test (12).

In conclusion, although a number of studies have demonstrated that sensitization to inhaled allergens is common in children with asthma, the relationship between childhood wheezing and sensitization to food allergens is not as clear. Sensitization to food allergens begins early in life and is strongly associated with symptoms of AD in infancy. It has also been reported that young children who are sensitized to food allergens, including those with or without AD, have an increased risk for developing allergic respiratory symptoms including asthma. Thus, it would be useful to know whether tests for foods could identify wheezing infants who are atopic, but who had not yet developed IgE ab to aeroallergens. In this study, however, IgE ab to food allergens was not a common finding in infants treated for wheezing in an emergency room. These children, in contrast to those infants referred to an allergy clinic for recurrent or persistent wheezing, also had a low prevalence of other risk factors for atopy. Among wheezing children ages 2 to 4, results from the emergency room revealed that tests for IgE ab to common food allergens may enhance efforts to identify atopic children who would not be identified by tests for inhaled allergens alone. After age

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4, most wheezing children with serum IgE ab had positive tests for inhalants and, in this age group, the data would support recommendations to test for IgE ab to food allergens in wheezing children more selectively, particularly in those who have a history of AD or food intolerance.

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REFERENCES

1. Nelson HS. The atopic diseases. *Ann Allergy*. 1985;55:441
2. Morrison-Smith J,Disney ME, William JP, Goels ZH. Clinical significance of skin test reactions to mite extracts in children with asthma. *Br Med J*. 1969;ii:723-726
3. Platts-Mills TAE, Tovey E, Mitchell E, Mozarro H, Nock P, Wilkins S. Reversal of bronchial reactivity during prolonged allergen avoidance. *Lancet*. 1982;2:675
4. Murray AB, Ferguson AC. Dust-free bedrooms in the treatment of asthmatic children with house dust or house dust mite allergy: a controlled trial. *Pediatr*. 1983;71:418
5. Ehnert B, Lau-Schadendorf S, Weber A, Buettner P, Schou C, Wahn U. Reducing domestic exposure to dust mite allergen reduces bronchial hyperreactivity in sensitive children with asthma. *J Allergy Clin Immunol*. 1992;90(1):135-138
6. Businco L, Benincori N, Cantani A: The spectrum of food allergy in infancy and childhood. *Ann Allergy*. 1986; 57:213-218
7. Novembre E, de Martino M, Vierucci A. Foods and respiratory allergy. *J Allergy Clin Immunol*. 1988;81(5):1059-1065
8. Sampson HA, James JM. Respiratory reactions induced by food challenges in children with atopic dermatitis. *Pediatr Allergy Immunol*. 1992;3(4):195-200
9. Minford AMB, Macdonald A, Littlewood JM. Food intolerance and food allergy in children: a review of 68 cases. *Arch Dis Child*. 1982;57:742-747
10. Bock SA. Respiratory reactions induced by food challenges in children with pulmonary disease. *Pediatr Allergy Immunol*. 1992;3(4):188-194

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

11. Onorato J, Merland N, Terral C, Michel FB, Bousquet J. Placebo-controlled double-blind food challenge in asthma. *J Allergy Clin Immunol.* 1986;78(6):1139-1146

12. Burks AW, Sampson HA. Diagnostic approaches to the patient with suspected food allergies. *J Pediatr.* 1992; 121: S64-S71.

13. Van Asperen PP, Kemp AS, Mellis CM. A prospective study of the clinical manifestations of atopic disease in infancy. *Acta Paediatr Scand.* 1984;73:80-85

14. Rowntree S, Cogswell JJ, Platts-Mills TAE, Mit/chell EB. Development of IgE and IgG antibodies to food and inhalant allergens in children at risk of allergic disease. *Arch Dis Child.* 1985;60:727-735

15. Zeiger RS. Prevention of food allergy in infancy. *Ann Allergy.* 1990;65:430-431

16. Buffum WP, Settipane GA. Prognosis of asthma in childhood. *Amer J Dis Child.* 1966;112:214-217

17. Pasternack D. The prediction of asthma in infantile eczema: a statistical approach. *J Pediatr* 1965; 66: 164-65

18. Stifler D. A 21 year follow-up of infantile eczema. *J Pediatr.* 1965; 66: 166-68

19. Langeland T. A clinical and immunological study of allergy to hen's egg white. *Clin Allergy.* 1983;13:371-382

20. Høst A, Halken S. A prospective study of cow milk allergy in Danish infants during the first 3 years of life. *Allergy.* 1990;45:587-596

21. Duff AL, Pomeranz ES, Gelber LE, et al. Risk factors for acute wheezing in infants and children: viruses, passive smoke, and IgE antibody to inhalant allergens. *Pediatr.* October,1993;(In press)

20283883331

Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

22. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol* 1980; 92(suppl):44-47.

23. Zeiger RS. Atopy in infancy and early childhood: natural history and role of skin testing. *J Allergy Clin Immunol.* 1985;75:633-639

24. Chapman MD, Platts-Mills TAE: Measurement of IgG, IgA and IgE antibodies to *Dermatophagoides pteronyssinus* by antigen binding assay, using a partially purified fraction of mite extract (F_4P_1). *Clin Exp Immunol.* 34: 126.

25. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72:248-254.

26. Kemeny DM, Richards D. Increased speed and sensitivity, and reduced sample size of a micro-radioallergosorbent test (RAST). *J Immunol Methods.* 1988;108:105-113

27. Eriksson NE. Total IgE influences the relationship between skin tests and RAST. *Ann Allergy.* 1989;63:65-69

28. Pollart SM, Chapman MD, Fiocco GP, Rose G, Platts-Mills TAE. Epidemiology of acute asthma: IgE antibodies to common inhalant allergens as a risk factor for emergency room visits. *J Allergy Clin Immunol.* 1989;83(5):875-882

29. Rosner B. *Fundamentals of biostatistics.* 3rd Edition, Boston, MA:PWS Kent Publishing Co.; 1990

30. Guillet G, Guillet MH: Natural history of sensitizations in atopic dermatitis. *Arch Dermatol* 1992; 128: 187-192.

31. Burks AW, Mallory SB, Williams LW, Shirrell MA. Atopic dermatitis: clinical relevance

20283883332

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of food hypersensitivity reactions. *J Pediatr* 1988; 113: 447-51.

32. Nagayama Y, Sakurai N, Nakahara T. Allergic predisposition among infants with bronchiolitis. *J Asthma.* 1987;24(1):9-17

33. Korppi M, Reijonen T, Pöysä L, Juntunen-Backman K. A 2- to 3- year outcome after bronchiolitis. *Amer J Dis Child.* 1993; 147:628-631

34. Taussig LM. The conundrum of wheezing and airway hyper-reactivity in infancy. *Ped Pul.* 1992;13:1-3

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Legends for Figures:

Figure 1:

The percentage of sera from wheezing patients (black bars) and controls (hatched bars) with IgE ab to food allergens are compared. In each serum, IgE ab was measured by RAST to egg, milk, soy, and peanut allergens. Sera testing positive to one or more of these allergens were defined as food RAST positive.

Figure 2:

The percentage of sera with IgE ab to egg (black bars), milk (slanted hatched bars), soy (open bars) and peanut (vertical hatched bars) are shown according to age for wheezing (A), control (B), and AD patients (C). The sample size of each age group is indicated in parentheses.

Figure 3:

Titers of IgE ab to egg (E), milk (M), soy (S), and peanut (P) were measured by RAST. The results in sera from AD, wheezing, and control patients are compared for children age 2 years and older. Titers are expressed as a percent of maximal counts bound of radiolabelled goat anti-human IgE ab at the top of a control curve established for each allergen. Solid circles (●) represent sera which were positive for IgE ab to each allergen. Numbers below the solid lines followed by an open triangle (△) represent the number of sera which tested negative.

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

Table 1. Patient Characteristics:

	CHILDREN <2			CHILDREN ≥2		
	Wheezing		AD	Wheezing		AD
	Patients	Controls	Patients	Patients	Controls	Patients
Number of patients	27	13	19	70	53	40
Mean age (yr)	0.9	1.2	1.1	7.0	9.9	5.6
Age range	0.3-1.8	0.5-1.8	0.5-1.9	2.0-15.4	2.3-16.1	2.0-13.0
Male (%)	56	27	84	66	64	52
Race: Black (%)	67	45	53	51	33	53
Family history (%)*	56	82	88	83	47	94
Total IgE†	15	4	109	166	27	541
(95% C.I.)	(9-26)	(2-10)	(47-253)	(110-251)	(18-42)	(288-912)
Payment Requirements:‡						
100%	15	58	47	18	31	40
10-75%	11	0	6	26	13	11
0%	73	42	47	56	56	49

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

Footnotes for Table 1:

* Analysis of the family history revealed a significantly increased prevalence of asthma, rhinitis, eczema and other allergic symptoms in immediate family members of wheezing and AD patients compared to controls after age 2 ($p < 0.001$ and $p < 0.001$, respectively).

† The total IgE levels in sera are expressed as geometric means in IU/ml. The 95% percent confidence intervals are also expressed in IU/ml.

‡ Payment requirement categories reflect the proportion of the medical bill charged to the patient's family based on annual family income. The percentages of families required to pay 100%, 10-75%, or 0% of their child's bill are shown.

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

TABLE 2: Positive RAST Responses to Food and Inhalant Allergens in Wheezing Children*

Sera with IgE ab to one or more:	Age < 2 y (n = 27)		Age 2 - 4 (n = 21)		Age ≥ 4 (n = 49)	
	Positive† Patients (%)	Total‡ IgE	Positive† Patients (%)	Total‡ IgE	Positive† Patients (%)	Total‡ IgE
Food Allergens§	1(4%)	59	5(24%)	140	15(31%)	217
Inhalant allergens‖	2(7%)	102	6(29%)	155	29(59%)	338
Foods only	0(0%)	--	3(14%)	137	5(10%)	55
Inhalants only	1(4%)	178	4(19%)	160	19(39%)	225
Foods and Inhalants¶	2(7%)	102	9(43%)	149	34(69%)	259

Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

Footnotes for Table 2:

* RAST data for IgE ab to five inhalant allergens (mite, cat, cockroach, ryegrass, and ragweed) were previously reported (18). Sera from 97 wheezing and 55 control patients were available from this study for measurements of IgE ab to food allergens. Sera from an additional 11 control patients were also analyzed.

† Number of RAST positive patients followed by the % who were positive in parentheses.

‡ Total serum IgE levels are reported as geometric means in IU/ml.

§ Sera with IgE ab to food allergens. Some also had IgE ab to inhalants.

‖ Sera with IgE ab to inhalants. Some also had IgE ab to foods.

¶ Total responses to food and inhalant allergens combined

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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

FIG. 1

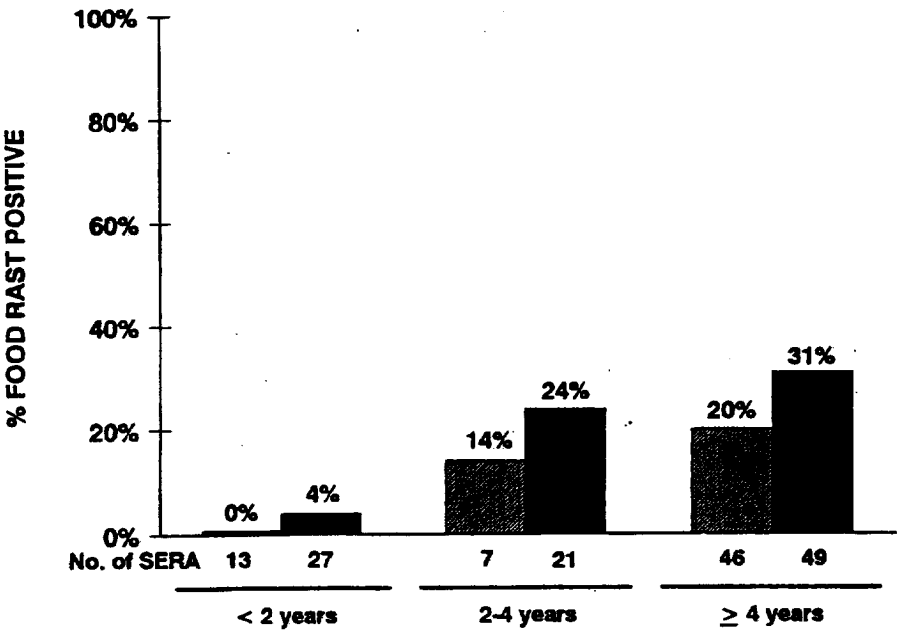
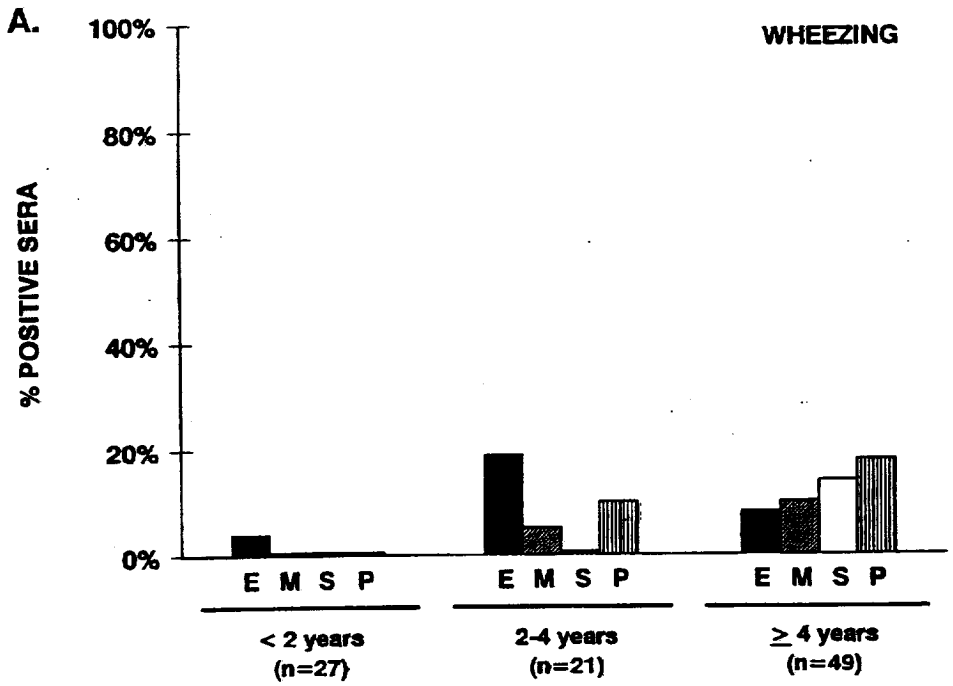


FIG. 2A



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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

FIG. 2B

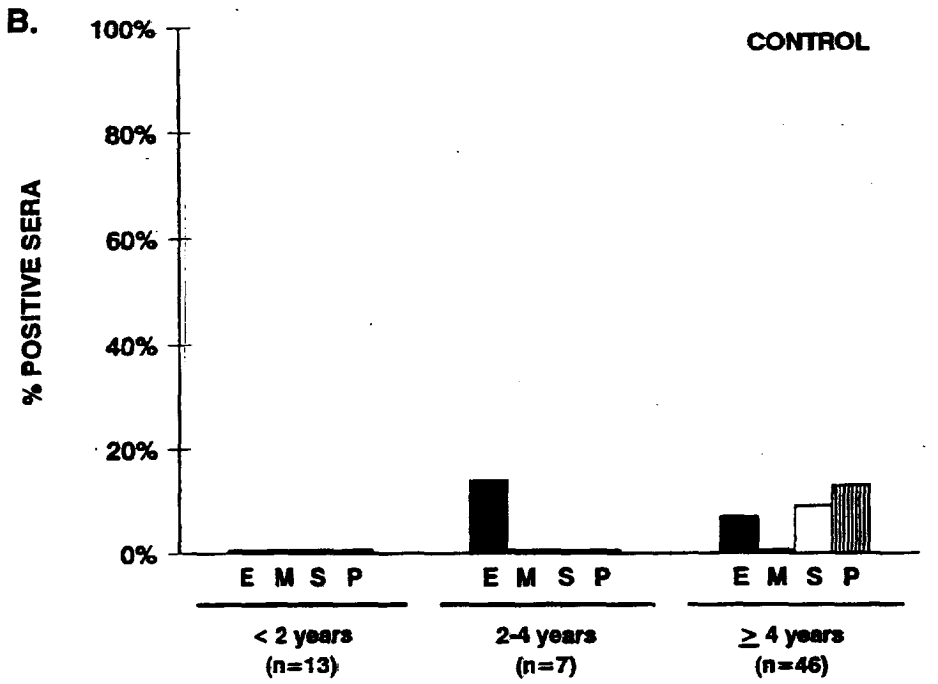
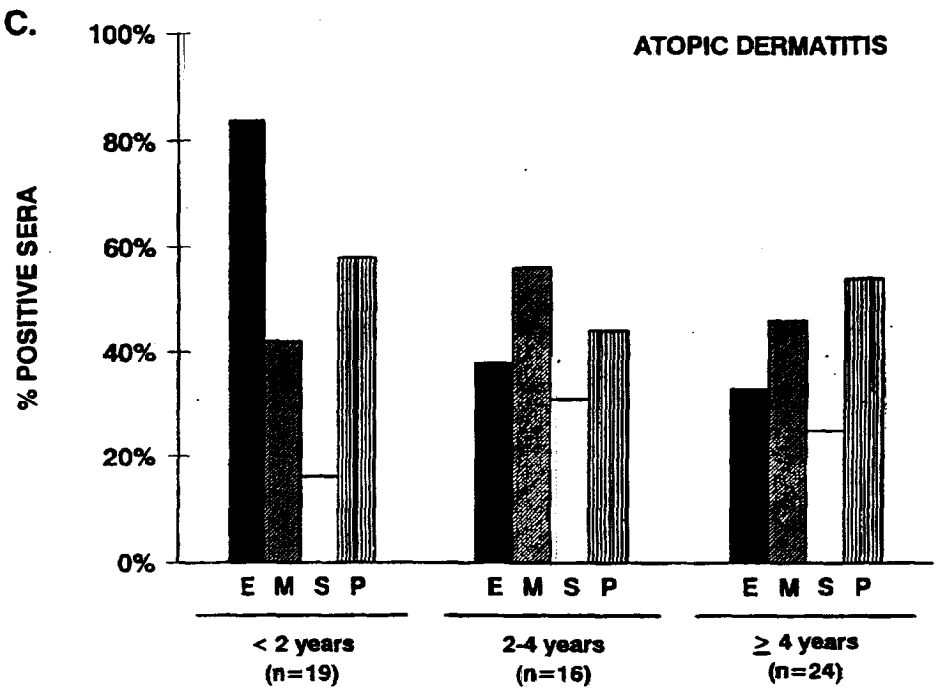


FIG. 2C



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Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000

FIG. 3

Source: https://www.industrydocuments.ucsf.edu/docs/pjnm0000